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Incidence of Bordetella bronchiseptica in swine and experimental production of rhinitis with the organism

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INCIDENCE OF BORDETELLA BRONCHISEPTICA IN SWINE AND EXPERIMENTAL PRODUCTION OF RHINITIS WITH THE ORGANISM

by

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INTRODUCTION

Atrophic rhinitis is a chronic infectious disease of the upper respiratory tract in swine. The most characteristic lesion of this disease is atrophy of the nasal turbinates. It affects a significant proportion of the swine raised in most areas of the world. The disease does not cause a high death loss, but interferes with growth and acts as an enhancing factor in pneumonias of swine.

The etiology of atrophic rhinitis has been uncertain for over a century. Nutritional deficiencies, genetic faults and infectious agents were incriminated by early workers. In the 1940's it was established that the disease was transmissible. Microorganisms found with some frequency in the porcine nasal cavity included trichomonads, Pseudomonas aeruginosa, Corynebacterium pyogenes, Mycoplasma hyorhinis, Pasteurella multocida and Hemophilus suis. Two of these organisms, Pasteurella multocida and Hemophilus suis, were shown in the 1950's to be capable of causing rhinitis and turbinate atrophy. A suspected viral rhinitis, reported in 1955, was characterized by the development of specific intranuclear inclusions and severe sneezing in young pigs (Done, 1955).

In 1956, Switzer demonstrated the multiple etiology of atrophic rhinitis. He reported that Pasteurella multocida, Bordetella bronchiseptica and a large filter passing agent were capable of producing rhinitis and turbinate atrophy.

Since 1956, 3 research groups have implicated Bordetella bronchiseptica as a cause of atrophic rhinitis (Switzer, 1959; Cross and Claflin, 1962; Goodwin and Whittlestone, 1963).

Acceptance of the concept that Bordetella bronchiseptica is a primary factor in atrophic rhinitis has been slow. There appear to be 2 reasons for this. First of all, Bordetella bronchiseptica, originally thought to cause canine distemper, has been relegated to the role of a minor pathogen since 1926 when it was conclusively demonstrated that the cause of that disease was a virus. Secondly, the etiologic factor of atrophic rhinitis was expected by some to be a new agent rather than agents which were common and easily detectible.

All species of the genus Bordetella are well established as etiologic agents of respiratory disease in man and animals. Bordetella pertussis causes whooping cough and Bordetella parapertussis causes a milder pseudowhooping cough in man. Bordetella bronchiseptica is a frequent secondary invader in canine respiratory disease, causes pneumonia in pigs, laboratory rats and guinea pigs and is a cause of chronic snuffles in domestic rabbits.

The similarity of clinical manifestations and histologic changes observed in diseases caused by members of the genus Bordetella indicate that their disease producing mechanisms are similar. Bordetella pertussis grows on the tracheal and bronchial mucosa in whooping cough. Virulence factors liberated by the bacterial cell are responsible for the development

of the local lesions and generalized symptoms seen in pertussis. Bordetella bronchiseptica grows on the tracheal and bronchial mucosa of infected animals. Virulence factors liberated by this organism are thought to be similar to those produced by Bordetella pertussis.

The need for elucidation of the role of Bordetella bronchiseptica in atrophic rhinitis was quite apparent at the inception of this study. It was known that the organism was capable of causing rhinitis in pigs, but its significance in the overall atrophic rhinitis problem was not known. It had been observed that several surgically repopulated swine herds had become infected with Bordetella bronchiseptica. Pigs in these herds were found to have severe lesions of turbinate atrophy from which the organism could be recovered in heavy growth.

The present study was undertaken to determine the incidence of Bordetella bronchiseptica in Iowa swine, to study the development of turbinate atrophy caused by this organism in respiratory disease-free pigs and to compare the relative pathogenicity for young pigs of several isolates of Bordetella bronchiseptica.

REVIEW OF LITERATURE

The review of literature is divided into various areas for convenience. These are entitled Classification; Morphologic and Growth Characteristics; Biochemic and Metabolic Activities; Serology, Antigens and Endotoxins; Heat Labile Toxins; Hemagglutinins; Hemolysins; Bacteriophages; Sensitivity to Antibacterial Agents; Pathogenicity of Bordetella Bronchiseptica; and Other Species of Bordetella.

Classification

The organism now known as Bordetella bronchiseptica was first named Bacillus bronchicanis by Ferry (1911). In 1912 he (Ferry, 1912a) changed the specific name to bronchisepticus since the organism had been found to infect several different species of animals. Ferry and Noble (1918) and Ferry and Klix (1918) demonstrated an antigenic relationship between Bordetella bronchiseptica and Bordetella pertussis. Evans (1918) showed that it was also antigenically related to Brucella abortus.

Bordetella bronchiseptica was classified in the genus Alcaligenes in the first edition of Bergey's Manual of Determinative Bacteriology (Bergey, 1923) because of its morphologic, growth and biochemic similarities to members of that genus. The close relationship of Bordetella bronchiseptica to Alcaligenes fecalis has been a subject of controversy until recent years. These 2 species are

peritrichously flagellated, Gram negative and somewhat pleomorphic. In addition, they both alkalinize litmus milk and carbohydrates and utilize citrate as a source of carbon. Neither species ferments carbohydrates. Isolates of both species are variable in their ability to reduce nitrates and produce similar reactions on potato medium.

Recent investigations by Szturm and Bourdon (1948), Ulrich and Needham (1953), Proom (1955) and Beer (1960b) indicated that Bordetella bronchiseptica produced positive urease reactions in less than 24 hours, required niacin and would not grow on simple glucose-inorganic salts media. Alcaligenes fecalis did not produce urease or require niacin for growth. It grew on simple glucose-inorganic salts media.

Beer (1960b) differentiated the 2 species on the basis of dye tolerance. Alcaligenes fecalis grew on agar containing 1:1000 thionin, 1:50,000 malachite green, 1:1000 crystal violet and 1:3000 methyl violet. Bordetella bronchiseptica was inhibited by these dyes at these levels. Both organisms grew in the presence of 1:3000 fuchsin.

Bordetella bronchiseptica was classified with the Brucellae in the fifth and sixth editions of Bergey's Manual of Determinative Bacteriology (Bergey et al., 1939; Breed et al., 1948). This classification was based on the biochemic, cultural, morphologic and antigenic similarities to members of that genus.

Wilson and Miles (1946, 1955) recognized the close

relationship of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica and placed all 3 species in the genus Hemophilus. For a time the name Haemophilus bronchiseptica enjoyed widespread usage in Europe.

Moreno-Lopez (1952) classified the 3 species in a new genus named Bordetella. Their antigenic, morphologic and biochemic similarities had been pointed out previously (Ferry and Klix, 1918; Eldering and Kendrick, 1938; Bruckner and Evans, 1939; Evans and Maitland, 1939; Eldering, 1941; Keogh et al., 1947). Evidence confirming the validity of this classification has been published in recent years (Andersen, 1953; Lacey, 1953a; Kendrick et al., 1953; Proom, 1953; Rauch and Pickett, 1961; Sutherland, 1961). Widespread acceptance of this classification is evident from the fact that it was used in both the seventh edition of Bergey's Manual of Determinative Bacteriology (Breed et al., 1957) and the fifth edition of Topley and Wilson's Principles of Bacteriology and Immunity (Wilson and Miles, 1964).

Morphologic and Growth Characteristics

McGowan (1911) and Torrey and Rahe (1913) reported that Bordetella bronchiseptica was a slender pleomorphic rod with rounded ends. Coccoid forms were found to be more common in tissues and on glycerin agar while bacillary forms were more common in broth cultures (McGowan, 1911). In addition, organisms from densely crowded colonies on agar medium were more

coccoid than organisms from larger isolated colonies (McGowan, 1911). McGowan (1911), Torrey and Rahe (1913) and Lautrop and Lacey (1960) found that coccoid forms of Bordetella bronchiseptica measured about 0.5 μ by 1.0 μ while bacillary forms measured about 0.5 μ by 1.5 μ in size. Torrey and Rahe (1913) reported that the 2 morphologic types of Bordetella bronchiseptica differed in their action on nitrates. The more coccoid type was found to reduce nitrates while the bacillary type did not.

Bordetella bronchiseptica was found to occur more frequently in pairs and chains in fluid media cultures (Ferry, 1911; Torrey and Rahe, 1913) and infected tissue (McGowan, 1911) than in solid media cultures. Torrey and Rahe (1913) reported that involution forms are found in older broth cultures, but not in cultures grown on solid medium. Evans (1918) observed that immune serum or pieces of tissue in the medium sometimes caused development of threads and clumps of bacterial cells.

McGowan (1911) and Torrey and Rahe (1913) found that Bordetella bronchiseptica appeared as minute circular dewdrops on solid medium after 24 hours of incubation at 37 C. They stated that during the next 24 hours the colonies grew quite rapidly and increased from an average size of less than a millimeter in diameter to several millimeters in diameter. These 48-hour colonies of Bordetella bronchiseptica were slightly convex, glistening, opalescent by reflected light

and translucent with a smoky tinge by transmitted light.

The growth of Bordetella bronchiseptica is quite characteristic on potato. Torrey and Rahe (1913) had the following comments:

> "In twenty-four hours at 37° C. a marked yellowish-brown growth always appeared with often a coincident greenish or grayish darkening of the medium. In two or three days the growth became typically raised, moist and copper to Van Dyck brown and the potato much darker. No further change occurred. The bacillus which most nearly simulates this type of growth on potato medium is B. fecalis alkaligenes, but, although the color is very similar, the growth is not raised and generally not so moist."

Similar reactions on potato were produced by Malleomyces mallei (Smith, 1913) and Brucella abortus (Smith, 1913; Evans, 1918).

Smith (1913) found that Bordetella bronchiseptica grew fairly well on gelatin at room temperature. He stated that liquefaction did not occur and that the growth varied with the type of gelatin. Winsser (1960) reported that foul-smelling, grayish colonies developed on chocolate agar. It has been reported that the organism grew well on MacConkey's agar (Goodwin and Whittlestone, 1962; Ross et al., 1963; Wilson and Miles, 1964), Endo agar (Winsser, 1960), desoxycholate agar (Winsser, 1960), and SS agar (Winsser, 1960). Winsser (1960) reported that it did not grow on bismuth sulfite agar.

Ferry (1911), McGowan (1911), Torrey and Rahe (1913), Smith (1913), Evans (1918) and Winsser (1960) described the

growth of Bordetella bronchiseptica in fluid medium. After 24 hours of incubation there was a cloudiness with a small amount of sediment. After longer incubation, tendrils from a thin pellicle were observed adhering to the wall of the tube. The sediment became ropy and the cloudiness remained unchanged. After several days a characteristic stale odor was noted.

Ferry (1911) and McGowan (1911) reported that Bordetella bronchiseptica was Gram negative. It was found to be bipolar when stained with carbolthionin (McGowan, 1911), Loeffler's methylene blue (Ferry, 1911; Torrey and Rahe, 1913) or toluidin blue (Torrey and Rahe, 1913). Smith (1913) stated that staining with strong carbolfuchsin followed by treatment with 0.1 percent acetic acid gave the sharpest and most distinct outlines.

Most workers reported that the organism did not have a capsule. However, Evans and Maitland (1939) reported that they had observed one. Repentigny and Frappier (1956) demonstrated a capsule in Bordetella bronchiseptica using flourescent antibody.

Bordetella bronchiseptica is motile by means of peritrichous flagella. Some authors describe it as actively motile (Ferry, 1911; Smith, 1913) while others state that motility can be easily overlooked in adapted strains (Torrey and Rahe, 1913; Winsser, 1960). Lacey (1953b) and Lautrop and Lacey (1960) observed that some strains of Bordetella

bronchiseptica were nonmotile. Winsser (1960) found that animal passage of sluggishly motile, adapted strains resulted in rejuvenation so that typical motility could be demonstrated.

Leifson (1960) reported that the flagella of 20 isolates of Bordetella bronchiseptica were uniform in their morphology. All isolates had peritrichous flagella and the mean wavelength of these flagella was 2.78 μ. In electron micrographs, palladium shadowed flagella of this organism were observed to have an "external contour of a counterclockwise or left-handed triple helix" (Labaw and Mosley, 1955). In addition, "the average periodicity along the length of the flagella was measured as 190 angstroms with the average diameter of the flagella measuring 139 angstroms." These workers estimated that the average length of Bordetella bronchiseptica flagella was 17 μ.

Biochemic and Metabolic Activities

McGowan (1911), Torrey and Rahe (1913), Smith (1913), Evans (1918) and Winsser (1960) reported that Bordetella bronchiseptica did not ferment carbohydrates, but produced a characteristic alkalinization of carbohydrate and litmus milk media. Torrey and Rahe (1913) made the following observations on the activity of this organism in litmus milk:

> "The reaction in this medium is very similar to that of B. fecalis alkaligenes. There is a progressive change to intense alkalinity. After twenty-four hours at 37° C. there appears a ring of deeper blue extending about three-eighths of an inch from the surface, varying in its intensity

> with different strains. After seventy-two hours the upper ring becomes intensely blue and the lower part of the tube much darker than the control. In from five to ten days the whole medium has assumed a blue black color. After fifteen days the lower fourth is found to be bleached and after three weeks the bleached part occupies the lower third of the medium. No further change was noted in cultures kept several weeks."

Bordetella bronchiseptica was found by Evans (1918) to decompose urea and asparagin with the production of ammonia. Other workers have emphasized the characteristic urease activity of this organism (Szturm and Bourdon, 1948; Ulrich and Needham, 1953; Lacey, 1953a; Beer, 1960b). Szturm and Bourdon (1948), Ulrich and Needham (1953), Proom (1955), Joubert et al. (1960) and Farkas-Himsley (1963) found that it utilized citrate as a source of carbon. Bradford and Slavin (1937), Rowatt (1957) and Lautrop and Lacey (1960) found that Bordetella bronchiseptica produced catalase. Lacey (1953a), Lautrop and Lacey (1960) and Farkas-Himsley (1963) reported that the organism produced cytochrome oxidase. Farkas-Himsley (1963) reported that it was lysine decarboxylase positive. Smith (1913), Torrey and Rahe (1913), Ferry (1911), Evans (1918) and Joubert et al. (1960) stated that it did not produce indole or hydrogen sulfide. It was not inhibited by peroxide (Rowatt, 1957) or colloidal copper sulfide (Proom, 1955). Most workers reported that some isolates reduced nitrates to nitrites while other isolates did not (Torrey and Rahe, 1913; Evans, 1918; Ferry and Noble, 1918; Spooner, 1938; Lautrop and Lacey, 1960). Ulrich and

Needham (1953), Proom (1955) and Lautrop and Lacey (1960) reported that Bordetella bronchiseptica required niacin for growth.

Serology, Antigens and Endotoxins

Dogs infected with Bordetella bronchiseptica were found to develop agglutinating antibodies by Ferry (1910), McGowan (1911), and Torrey and Rahe (1913). Circulating antibody titers in infected animals reached 1:2000 (Ferry, 1911), but titers of supposedly normal dogs ranged from 1:10 to 1:500 (Torrey and Rahe, 1913).

In 1927 Bailey reported that serum from rabbits infected with Bordetella bronchiseptica had high titers of agglutinating but not complement fixing antibodies. Bull and McKee (1928) showed that Bordetella-free rabbits immunized with a bacterin and subsequently infected with Bordetella bronchiseptica remained positive for the organism even when the agglutinating antibody titer was quite high. In addition, they found that rabbits previously unexposed to the organism developed high titers after exposure.

Ferry (1911) detected some variability in the antigenic composition of different strains of Bordetella bronchiseptica. However, McGowan (1911) and Torrey and Rahe (1913) felt that no significant variability occurred. Eldering (1941) observed that laboratory passage of Bordetella bronchiseptica frequently resulted in the development of rough colony forms that

were poorly antigenic, nonpathogenic and nonhemolytic. Similar observations were made by Flosdorf et al. (1941). Beer (1960b) observed the development of smooth, intermediate, and rough colony forms. Lacey (1953a, 1953b) found that alteration of the ionic composition of the growth medium or the incubation temperature resulted in reversible changes in the antigenic structure of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica.

In 1918 Bordetella bronchiseptica and Bordetella pertussis were shown to have common antigens by means of agglutination (Ferry and Noble, 1918) and complement fixation tests (Ferry and Klix, 1918). Leslie and Gardner (1931) confirmed this finding. The antigenic relationship of Bordetella pertussis, Bordetella bronchiseptica and Bordetella parapertussis has been studied by other workers (Eldering and Kendrick, 1938; Bruckner and Evans, 1939; Evans and Maitland, 1939; Flosdorf et al., 1941; Andersen, 1953; Eldering et al., 1957; Winsser, 1960; Sutherland, 1961). All 3 species are now considered to have common species specific K or heat labile antigens and common O or heat stable antigens (Andersen, 1953; Eldering et al., 1957; Lautrop and Lacey, 1960). Bordetella bronchiseptica was also reported to have H or flagellar antigens (Lautrop and Lacey, 1960).

Eldering (1941, 1942) isolated lipopolysaccharides from strains of the 3 Bordetella species. The preparation obtained from Bordetella bronchiseptica was toxic for mice. Similar

preparations from all 3 species were found to be capable of immunizing mice against intraperitoneal challenge with Bordetella bronchiseptica. Maclennan (1960) reported the use of a phenol extraction procedure to isolate specific lipopolysaccharides from each of the 3 species. These preparations were found to contain an aldoheptose, a hexose and a hexosamine. Sutherland (1961) confirmed these findings. Maclennan (1960) found that these lipopolysaccharides were active in serological tests and differed in their serological specificity. He reported that their chemical and biological properties were similar to those of lipopolysaccharides prepared from other Gram negative bacteria. He also found that lipopolysaccharides prepared from avirulent strains of Bordetella bronchiseptica and Bordetella pertussis differed from those prepared from virulent strains.

Heat Labile Toxins

Torrey and Rahe reported in 1913 that filtrates of broth cultures of Bordetella bronchiseptica administered intraperitoneally killed guinea pigs. They attempted to produce an antitoxin but were unsuccessful.

Evans and Maitland (1939) found that extracts of Bordetella bronchiseptica were similar to those prepared from Bordetella pertussis in both lethal activity in guinea pigs and dermonecrotic activity in rabbits. Oddy and Evans (1940) demonstrated that extracts of these 2 species were capable

of inducing hyperglycemia followed by hypoglycemia when injected intravenously in rabbits.

The extracts prepared by Evans and Maitland (1939) decreased in potency when filtered through a Seitz E K filter and were less stable when stored at room temperature than at 5 C. They could be stored in a dried state at 5 C. for up to 4 months. These preparations were sensitive to formalin and were nonantigenic.

The heat labile toxin of Bordetella pertussis is now thought to be a protein of cytoplasmic origin. Banerjea and Munoz (1962) and Billaudelle et al. (1960) found that the dermonecrotic and lethal factors could be separated by electrophoresis and chromatography and noted that the dermonecrotic factor was more stable than the lethal factor. It has been speculated that this toxin may be responsible for the toxicity of fresh whole-cell pertussis vaccines (Munoz, 1963).

Hemagglutinins

Keogh, et al. (1947) demonstrated that saline suspensions of Bordetella bronchiseptica, Bordetella pertussis and Bordetella parapertussis agglutinated human, mouse and fowl erythrocytes. Filtrates and supernatants of broth cultures of these organisms showed the same activity. Virulent strains of Bordetella bronchiseptica were rich in hemagglutinin while avirulent strains produced little hemagglutinin and were

nonhemolytic. The hemagglutinating activity could be neutralized with specific immune serum. There was a correlation between the protective antibody and the antihemagglutinin activity of this serum. However, the agglutinating and antitoxic antibody of immune serum were distinct from the antihemagglutinin.

Masry (1952) found that the hemagglutinin of Bordetella pertussis could be extracted from young agar grown cells with solutions of sodium chloride or sodium acetate. He also observed that the hemagglutinin was most potent in the early phases of growth in fluid media. Joubert et al. (1960) found that Bordetella bronchiseptica agglutinated sheep and human erythrocytes but not horse erythrocytes.

Hemolysins

McGowan (1911) and Winsser (1960) observed that incorporation of blood in agar medium stimulated the growth of Bordetella bronchiseptica. It has been found to be beta hemolytic on sheep (Winsser, 1960; Ross et al., 1963), rabbit (Torrey and Rahe, 1913; Winsser, 1960; Ross et al., 1963), dog (Torrey and Rahe, 1913), guinea pig (Torrey and Rahe, (1913), horse (L'Ecuyer et al., 1961a; Ross et al., 1963) and bovine (Ross et al., 1963) blood agar. Eldering (1941), Winsser (1960), and L'Ecuyer et al. (1961a) reported that freshly isolated strains were more hemolytic than laboratory adapted strains. L'Ecuyer et al. (1961a) found that passage

of the organism in the allantoic sac of embryonating hen's eggs resulted in increased hemolysis on 5 percent horse blood agar.

Winsser (1960) found that no hemolysis occurred when fresh, washed, rabbit, sheep or human erythrocytes were incorporated in broth cultures of Bordetella bronchiseptica. He pointed out that hemolysis on agar medium induced by Bordetella bronchiseptica was a "hazy incomplete clearing" which was produced inconstantly. It was most pronounced where many colonies were close together and was usually absent where the colonies were few in number. He stated that it showed up most clearly after "hot-cold" incubation.

Bacteriophages

Rauch and Pickett (1961) reported the isolation of 38 bacteriophages from 48 isolates of Bordetella bronchiseptica. All phages appeared to be spontaneously inducible. Each of 9 isolates of Bordetella parapertussis was sensitive to at least 2 of the Bordetella bronchiseptica phages. Fifty isolates of Bordetella pertussis examined were not sensitive to any of the phages. In addition, 7 isolates of Alcaligenes fecalis and several isolates of Brucella spp. were insensitive to these phages.

Sensitivity to Antibacterial Agents

The sensitivity of Bordetella bronchiseptica to certain physical and chemical agents was studied by Torrey and Rahe

(1913). They found that this organism was unusually sensitive to moist heat. Twenty-four hour broth cultures were killed in 5 to 10 minutes at 55 C. Dry heat killed the organism in less than 5 minutes at 100 C., however, it was extremely resistant to drying at room temperature. Cultures dried over calcium chloride lived up to 5 months at room temperature. Twenty-four hour broth cultures of Bordetella bronchiseptica were found to be fairly resistant to repeated freezing and thawing. The organism was more resistant to exposure to direct sunlight than Salmonella typhosa, Shigella dysenteria, Pseudomonas aeurginosa, Escherichia coli and Vibrio cholera. Switzer[1] found that tryptose phosphate broth cultures of this organism were still viable after at least 9 years of incubation at 37 C.

Torrey and Rahe (1913) also determined the sensitivity of the organism to several germicides. Mercuric chloride was found to be particularly effective. Repeated intranasal instillation of mercurochrome cleared Bordetella bronchiseptica from the nasal cavities of infected rabbits (Bull and Bailey, 1927).

Bordetella bronchiseptica was sensitive in vitro to chlortetracycline (Winsser, 1960; L'Ecuyer et al., 1961a; Switzer, 1963), oxytetracycline (L'Ecuyer et al., 1961a),

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[1]Switzer, W. P., Veterinary Medical Research Institute, Iowa State University, Ames, Iowa. Studies on atrophic rhinitis. Private communication. 1962.

tetracycline (Winsser, 1960; L'Ecuyer et al., 1961a), chloramphenicol (Joubert et al., 1960; L'Ecuyer et al., 1961a), sulfaethoxypyridazine (Switzer, 1963) and sulfamethazine (Switzer, 1963). Slavin and Maclay (1947) found that the organism was more sensitive to sulfapyridine, sulfathiazole, sulfadiazine or sulfamerazine than to sulfamethazine or sulfanilamide. Joubert et al. (1960) found the organism highly sensitive in vitro to sulfamide and sulfamethiazole. It was slightly less sensitive to sulfathiazole, sulfadiazine and sulfamerazine. L'Ecuyer et al. (1961a) found the organism resistant to furacin and furadantin, but Joubert et al. (1960) reported that it was highly sensitive to furazolidone. It was only slightly sensitive to streptomycin (Joubert et al., 1960; L'Ecuyer et al., 1961a), erythromycin (L'Ecuyer et al., 1961a) and novobiocin (Winsser, 1960) and was resistant to penicillin (Joubert et al., 1960; L'Ecuyer et al., 1961a).

Observations on pneumonia in rats due to Bordetella bronchiseptica revealed that streptomycin, chloramphenicol, chlortetracycline and oxytetracycline were effective medications (Rosen et al., 1954). Sulfamethazine and neoprontisil reduced the death rate, but were not as effective as the antibiotics. Penicillin was ineffective.

Swine with pneumonia caused by this organism responded to treatment with chlortetracycline (L'Ecuyer et al., 1961a). However, use of this antibiotic in treatment of rhinitis caused by Bordetella bronchiseptica was not beneficial

(Switzer, 1963). Sulfamethazine (Switzer, 1963) and sulfathiazole[1] have been found to be highly effective therapeutic agents in the treatment of swine with nasal Bordetella bronchiseptica infection.

Pathogenicity of Bordetella Bronchiseptica

Bordetella bronchiseptica in swine

Bordetella bronchiseptica and organisms resembling it have been isolated from normal and pneumonic swine lungs since 1922 (Dorset et al., 1922; Spray, 1922; Thorp and Tanner, 1940; Phillips, 1943 and 1944; Genest, 1944; Morcas et al., 1947; Betts, 1952; Ryu, 1954; Beer, 1960a; L'Ecuyer et al., 1961b; Goodwin and Whittlestone, 1964). The first recognition of Bordetella bronchiseptica as a significant primary cause of pneumonia in swine was published by Phillips (1943). He isolated the organism from the bronchial exudate of pigs from several farms in Ontario. The clinical disease was characterized by coughing and unthriftiness. Ray reported the same disease in the United States (1950, 1959). He found that few pigs died in the acute stages, but that most infections became chronic.

Several reports of outbreaks of Bordetella bronchiseptica pneumonia in young pigs have appeared in recent years (Joubert

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[1]Switzer, W. P., Veterinary Medical Research Institute, Iowa State University, Ames, Iowa. Studies on atrophic rhinitis. Private communication. 1963.

et al., 1960; L'Ecuyer et al., 1961a; Dunne et al., 1961; Goodwin and Whittlestone, 1962). Experimental production of the disease was reported by L'Ecuyer et al. (1961a) and Goodwin and Whittlestone (1962). L'Ecuyer et al. (1961a) described the gross character of pneumonia in swine due to Bordetella bronchiseptica. The early lesions were reddish tan in color and quite firm. After about 2 weeks duration these lesions were grayish-yellow in color and very firm and fibrous on palpation. In addition there were small purple foci scattered throughout the involved tissue. Cross sections revealed numerous brownish necrotic foci. The histopathology of this pneumonia has been studied (L'Ecuyer et al., 1961a; Dunne et al., 1961; Roberts et al., 1962). Phillips (1944) and Ray (1950, 1959) recommended the use of bacterins in the control of the disease in infected herds.

The first report of Bordetella bronchiseptica in the porcine nasal cavity was that of Daugherty (1941). He stated that this organism had been isolated from the nasal cavities of pigs with rhinitis. This report predated the first report of atrophic rhinitis in the United States made by Doyle et al. (1944). Phillips (1946) listed Bordetella bronchiseptica as a secondary invader in atrophic rhinitis. In 1947 Moynihan isolated the organism from the nasal cavities of 2 pigs with atrophic rhinitis. He made the following statement:

> "Staphylococcus albus was isolated from the nasal passages of each affected pig and Pseudomonas aeruginosa, Corynebacterium pyogenes, Staphylococcus

> aureus and Alcaligenes bronchisepticus were isolated from two of the pigs. All of these organisms at one time or another have been suspected of causing rhinitis."

Moynihan transmitted broth cultures of Bordetella bronchiseptica, Staphyloccus albus and Staphylococcus aureus to a normal 3-month-old pig. After 39 days there was no evidence of rhinitis.

Switzer (1956, 1959, 1964) proposed the multiple etiology concept of atrophic rhinitis. He presented evidence that filter-passing agents, Bordetella bronchiseptica and Pasteurella multocida, were capable of inducing rhinitis and turbinate atrophy in swine. Claflin (1958) reported that pure cultures of Alcaligenes fecalis caused turbinate atrophy. It is probable that his organism was Bordetella bronchiseptica.

Dunne (1961) reported the isolation of Bordetella bronchiseptica from the nasal cavity of a pig with Bordetella bronchiseptica pneumonia. Goodwin and Whittlestone (1962, 1963) reported producing rhinitis as well as pneumonia with broth cultures of the organism. Cross and Claflin (1962) produced rhinitis and turbinate atrophy of swine with broth cultures of Bordetella bronchiseptica. In addition, they isolated the organism from 9 of 10 pigs submitted from 10 separate field outbreaks of atrophic rhinitis.

Part of the experimental work contained in this dissertation was published in 1963 (Ross et al., 1963a). It was found that 10 of 15 pigs infected at 4 weeks of age with Bordetella

bronchiseptica and necropsied from 2 to 8 weeks postinoculation had mild to moderate turbinate atrophy. In addition, 15 of 16 pigs infected with the organism at 3 days of age and necropsied from 2 to 5 weeks postinoculation had mild to severe turbinate atrophy. Bordetella bronchiseptica was found to be extremely common in the nasal cavities of Iowa swine (Ross et al., 1963b; Ross, 1964).

Bordetella bronchiseptica in guinea pigs

Several reports in the early literature on guinea pig pneumonia contain reference to organisms which could have been Bordetella bronchiseptica (Tartakowsky, 1897; Martini, 1900; Wherry, 1902; Selter, 1906). McGowan (1911) reported that he had isolated the organism from the tracheas of normal guinea pigs. Ferry (1912b, 1914) reported that Bordetella bronchiseptica was a cause of pneumonia in guinea pigs and a common cause of death losses in guinea pigs in shipments from animal suppliers. He isolated the organism from the tracheas, lungs, blood, internal organs and intestines of these guinea pigs. He suggested using a bacterin to control the disease.

Theobald Smith (1913) reported that pneumonia had been encountered repeatedly in guinea pigs in his laboratory since 1899. Culture of the lung tissue from these animals revealed pure growth of Bordetella bronchiseptica in 90 percent of the cases. Cultures of the spleens, livers and kidneys were uniformly negative. Occasionally, the organisms were isolated

from the uterus. Dead embryos were usually present in such uteri. He found the organism in the bronchi of a few healthy guinea pigs, but felt that most normal guinea pigs were negative. He speculated that seemingly normal animals that were positive for the organism may have survived a pneumonia outbreak.

Smith observed that gross lesions produced by Bordetella bronchiseptica consisted of varying degrees of hepatization in the anterior lobes of the lungs. The involved tissue varied from a uniform flesh red to a variegated grayish red. The gray lesions were always very firm. He observed the bacilli attached to the cilia of the bronchial mucosa in histologic sections. He found that administration of cultures of the organism per os, intranasally and intravaginally resulted in no lesions. This was in contrast to the report by Winsser (1960) who found that intranasal inoculation of Bordetella bronchiseptica resulted in chronic nasal infection or death due to pneumonia.

Bordetella bronchiseptica was isolated from the pneumonic lungs of guinea pigs and tracheas of healthy guinea pigs by Evans (1918). She also isolated the organism from the spleen, heart blood, kidneys and uteri of these animals. Keegan (1920) isolated Bordetella bronchiseptica from the lungs and tracheas of guinea pigs with pneumonia. He found that in necropsies of animals from an infected colony over 50 percent had some lung lesions. Bordetella bronchiseptica was

frequently present in the tracheas of these animals. Griffin (1955), Beer (1960a), Dunne et al. (1961), and Hagan and Bruner (1961) listed Bordetella bronchiseptica as an important cause of pneumonia in guinea pigs.

Bordetella bronchiseptica in dogs

Ferry (1910) reported the isolation of an organism now known as Bordetella bronchiseptica from the lungs, bronchi, tracheas, heart blood and small intestines of dogs with canine distemper. In 1911 McGowan, working independently, reported the isolation of a similar organism from distemper dogs and other animals with respiratory diseases. Pure cultures of Bordetella bronchiseptica were reported by these workers to be capable of causing canine distemper in susceptible dogs. In addition, both workers believed they were able to prevent the disease by immunizing dogs with killed cultures of Bordetella bronchiseptica.

Torrey and Rahe (1913) found the organism in pure culture in the trachea in 80 percent of 90 dogs with canine distemper. They also found it in the nasal exudate 50 percent of the time and in a few cases were able to isolate it from the liver, kidney, spleen and heart blood. In one case it was isolated from the conjunctiva.

Torrey and Rahe (1913) were able to produce a serious respiratory disease in dogs with cultures of the organism. They were also able to protect dogs against the disease by

administration of a bacterin. They made several interesting observations on the effects of other bacteria on Bordetella bronchiseptica:

> "We come now to the discussion of a locality most favorable for B. bronchisepticus and that is the respiratory tract, especially the trachea. This bacillus is markedly aerobic and also resists weakly the antagonism of other bacteria."
>
> "Given a clear, well-aerated field, as in the lower respiratory tract, and it grows luxuriantly, but following the advent of secondary invading bacteria, this bacillus may be crowded out with greater or less rapidity, first from the nose and later from the entire respiratory tract."

Rhea (1915) reported Bordetella bronchiseptica tended to localize between the cilia of the tracheal and bronchial epithelium of infected dogs. He also observed that Bordetella bronchiseptica produced lesions in dogs similar to those produced by Bordetella pertussis in man.

Lockhart (1927) reported that Bordetella bronchiseptica, staphylococci and streptococci were commonly isolated from lesions in the respiratory tracts of dogs. Schlingman (1931) recovered Bordetella bronchiseptica from the respiratory tracts of 93 percent of 72 dogs with canine distemper. Bordetella bronchiseptica was isolated from 12 of 200 surgically removed canine tonsils (Smith, 1940).

In studies on kennel cough, Greig (1954) found that a canine mycoplasma and freshly isolated Bordetella bronchiseptica in combination or alone would not produce respiratory

disease in experimental dogs. Mosier (1955) stated that Bordetella bronchiseptica and Streptococcus canis were frequently isolated from the throats of dogs with kennel cough. However, he was unable to reproduce the disease with the organisms.

Merchant and Packer (1961) and Hagan and Bruner (1961) list Bordetella bronchiseptica as a frequent secondary invader in canine distemper and other canine respiratory diseases.

Bordetella bronchiseptica in rabbits

Bordetella bronchiseptica was found in a high percentage of the tracheas and nasal cavities of 25 rabbits by McGowan (1911). Many of these rabbits had a clinical rhinitis. Ferry (1912b) reported an epizootic of respiratory disease caused by this organism. He emphasized that the organism was easily isolated from the trachea. In 1914 he (Ferry, 1914) suggested using a bacterin in the control of the disease.

Ferry and Hoskins (1920) reported that Bordetella bronchiseptica was a cause of snuffles in laboratory rabbits. They isolated the organism from the nasal cavities, sinuses and tracheas of a large number of rabbits. They suggested sanitation and the use of a bacterin to aid in control of the disease.

Webster (1924a, 1924b) incriminated Pasteurella multocida as the causative agent of rabbit snuffles. He reported that this organism was present more consistently and in greater

numbers than other bacteria isolated from rabbits with snuffles. In addition, he showed that 30 to 40 percent of normal rabbits as well as diseased rabbits had Bordetella bronchiseptica in their nasal cavities. Pasteurella multocida was far less common in the nasal cavities of normal rabbits. Smith and Webster (1925) infected 16 Bordetella bronchiseptica-free rabbits with the organism. At necropsy all 16 had become nasal carriers and 4 had the organism in the middle ear with some evidence of otitis media. Tanaka (1926) reported that Bordetella bronchiseptica could occasionally be isolated from cases of sinusitis and rhinitis in rabbits. He felt that Pasteurella multocida was more frequently associated with these lesions.

In 1927 Bailey presented evidence that rabbits infected with Bordetella bronchiseptica frequently have high titers of circulating agglutinating antibody. In his experience, rabbits with the highest agglutinating antibody titers had some of the heaviest nasal infections. It was reported that repeated intranasal instillation of mercurochrome cleared Bordetella bronchiseptica from the nasal cavities of 17 of 19 carrier rabbits (Bull and Bailey, 1927).

Bull and McKee (1928) found that pneumococci seeded on the nasal mucosa of nonimmune rabbits survived and multiplied for 4 to 5 days. Rabbits which had been previously exposed to pneumococci or immunized with a bacterin were able to shed the organism as early as 4 hours after infection. In contrast,

rabbits immunized with a Bordetella bronchiseptica culture were found to carry this organism indefinitely in their nasal cavities in spite of high titers of circulating antibody.

Andersen (1953) stressed the importance of Bordetella bronchiseptica in rabbits used in pertussis studies. She stated that rabbits infected with this organism frequently had antibodies that reacted with Bordetella pertussis. In 1964 Hagen[1] observed that about 20 percent of several hundred normal rabbits were carrying Bordetella bronchiseptica in their nasal cavities.

Bordetella bronchiseptica has been isolated from the pneumonic lungs of laboratory rabbits (McGowan, 1911; Rosenow, 1931; Hagan, 1958; Smith and Webster, 1925). In 1945 Kyaw reported the isolation of a Bordetella bronchiseptica-like organism from the genital tracts of doe rabbits with infertility problems.

Several other authors list Bordetella bronchiseptica as a common inhabitant and frequent pathogen of the rabbit respiratory tract (Griffin, 1955; Sanford et al., 1957; Ostler, 1961; Hagan and Bruner, 1961).

Bordetella bronchiseptica in rats

Borden and Kulp (1939) isolated Bordetella bronchiseptica from the lungs and heart blood of rats with acute pneumonia.

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[1]Hagan, K. W., U. S. Rabbit Experiment Station, Fontana, California. Observations on rabbit snuffles. Private communication. 1964.

They reproduced the disease by intratracheal inoculation of the organism. Nelson (1940) isolated the organism from the lungs of 1 rat and from the nasal cavity of a second rat.

An epizootic of Bordetella bronchiseptica pneumonia killed 250 of 1000 laboratory rats (Rosen et al., 1954). The authors found that terramycin in the feed at a level of 0.1 percent effectively controlled the outbreak. In a second paper they reported the successful use of a bacterin as an immunizing agent in their colony (Wickert et al., 1958). Sacquet (1959) reported that he had observed a disastrous outbreak of pneumonia in a laboratory colony of rats caused by Bordetella bronchiseptica.

Winsser (1960) stated that Bordetella bronchiseptica had been isolated from a wild rat. This isolation probably was made from the respiratory tract. He also reported a pneumonia outbreak in a colony of laboratory rats in which several died. The organism was isolated from the lungs and middle ear of an affected rat. Intranasal inoculation of a suspension of pneumonic lung from this rat into 4 young rats did not result in illness. At necropsy 2 months later, 2 rats had lesions of pneumonia and 3 had lesions of otitis media. Bordetella bronchiseptica was recovered from the lungs of all 4 rats. Similar results were obtained when a broth culture was given intranasally to 20 young, pathogen-free rats. He felt that his results were consistent with the concept that laboratory rats under good conditions are resistant to clinical

disease, but frequently develop a carrier state.

Nelson (1960), Beer (1960a) and Hagan and Bruner (1961) listed Bordetella bronchiseptica as a cause of pneumonia in rats.

Bordetella bronchiseptica in humans

The first human infection with Bordetella bronchiseptica was reported by McGowan (1911). A laboratory worker who frequently handled rabbits and guinea pigs had suffered a chronic rhinitis with acute exacerbatio s for about 18 months. A pure culture of the organism was isolated from pus present on the soft palate. Ferry (1913) also studied a human isolate of Bordetella bronchiseptica.

Brown (1926) reported a case of whooping cough due to Bordetella bronchiseptica in a 5-year-old girl. The girl had received a pet rabbit for Easter. The rabbit developed symptoms of snuffles. Ten to 12 days after Easter the girl began to cough at night. This developed into the typical paroxysms of whooping cough. Bordetella bronchiseptica was isolated from her throat and from the nares of the rabbit. After about 1 month the coughing subsided. Evans and Maitland (1939) quote Scott as stating that he found Bordetella bronchiseptica not infrequently present in human infections including meningitis.

Man (1950) isolated Bordetella bronchiseptica from a 2-year-old child. The child had been immunized against

pertussis at 1 year of age and had intimate contact with dogs. The early symptoms of sneezing and rhinorrhea gradually progressed to typical paroxysmal coughing. One month after hospitalization the patient was asymptomatic, but a nasal pharyngeal culture was still positive.

Mention was made in the 1951 report of the British Medical Research Council of a case of whooping cough due to Bordetella bronchiseptica in which the child coughed for 6 weeks. Kendrick et al. (1953) record a communication from Alexander in which she reported isolating Bordetella bronchiseptica from 2 children with whooping cough-like diseases. Lacey (Lautrop and Lacey, 1960) estimated that 0.1 percent of the cases of whooping cough in London were due to Bordetella bronchiseptica.

Winsser (1960) isolated the organism from the throat of a caretaker who worked with animals which were thought to be negative for the organism. The individual's household contacts and animals at home were also negative. This individual had suffered a bronchopneumonia 18 months previously and had recurrent colds and an occasional "croup-like", nonproductive cough, resembling mild whooping cough.

An outbreak of Bordetella bronchiseptica infections in which both children and family pets were involved was described in 1962 (Veterinary Dispatch, 1962). The outbreak started as a respiratory disorder in the pet rabbits and cats. The 6 youngest of the 11 children subsequently developed a

respiratory disease. Two of these showed definite signs of whooping cough. Bordetella bronchiseptica was isolated from all 6 children, a surviving cat and a kitten. The older children, the parents, a dog and a puppy were negative.

Bordetella bronchiseptica in other species of animals

McGowan (1911) reported an endemic of Bordetella bronchiseptica infection in a colony of laboratory cats. Cats entering the colony were negative on nasal culture. After a few weeks most became nasal carriers and many were found to have tracheal infections. The organism was isolated from the pneumonic lungs of 20 of these cats and from the conjunctiva of 1 cat. Scott et al. (1957) mention isolating Bordetella bronchiseptica from the mouth, nasal and ocular discharges of unhealthy kittens.

McGowan (1911) isolated Bordetella bronchiseptica from the respiratory tracts of 2 ferrets. Spooner (1938) observed distemper in ferrets in which canine distemper virus and Bordetella bronchiseptica were involved. Ferrets inoculated with the virus alone rarely developed pneumonia, but the virus and the bacterium in combination frequently resulted in pneumonia. Intranasal inoculation of the bacterium alone resulted in only a transient pyrexia. Winsser (1960) reported that rhinitis and respiratory distress developed in 2 ferrets infected intranasally with Bordetella bronchiseptica. One animal died. Focal abscesses were found in the lung and the

organism was recovered from these lesions.

Bordetella bronchiseptica was isolated from the respiratory tracts of monkeys by McGowan (1911) and Ferry (1912b). Winsser (1960) isolated the organism from the respiratory tracts of 11 monkeys. All of the animals had lesions of pneumonia. He experimentally infected a monkey by intranasal inoculation. One month later this monkey had no pneumonic lesions, but the organism was recovered from the trachea and bronchi.

Keegan (1920) reported an epidemic of pneumonia in mice and guinea pigs. Bordetella bronchiseptica was isolated from the lungs of 6 of 24 mice examined. Evans and Maitland (1939) stated that mice generally do not harbor Bordetella bronchiseptica. However, these workers were able to produce an experimental pneumonia in white mice by inoculation of this organism. The organism was isolated from pneumonic mouse lungs by Ratcliffe (1945). Griffin (1955) stated that the organism may spread from infected guinea pigs to mice housed in close proximity. Bacon et al. (1958) isolated Bordetella bronchiseptica from the liver and spleen of a vole.

Edwards (1957) noted that Bordetella bronchiseptica is a common inhabitant of the respiratory tract of the European hedgehog. Under the stress of captivity the organism frequently causes pneumonia and rhinitis in this animal.

Bordetella bronchiseptica has been isolated from foxes with canine distemper (Rosenow, 1931; Pinkerton, 1940).

McGowan (1911) isolated it from the trachea and pneumonic lung of a goat. Winsser (1960) found that the hamster is quite refractory to experimental infection with this organism.

Other Species of Bordetella

Excellent reviews of the literature on Bordetella pertussis have been published by Lautrop and Lacey (1960), Billaudelle et al. (1960), Sutherland (1961) and Munoz (1963). Only the most pertinent factors regarding this organism will be discussed.

Bordetella pertussis was first recovered from cases of whooping cough by Bordet and Gengou in 1906. They were able to grow it on a potato glycerin agar containing 50 percent mammalian blood. Modifications of this medium are still employed in many laboratories for the isolation of Bordetella pertussis. The organism is more fastidious in its growth requirements than Bordetella parapertussis or Bordetella bronchiseptica.

Bordetella pertussis is a pleomorphic Gram negative rod. Virulent strains are typically coccobacillary and uniform in size. It is nonflagellated and has a capsule. Bordetella pertussis has several antigenic substances which are characterized by certain biologic properties. The cell wall has been shown to contain a protective antigen and a histamine sensitizing factor (Munoz et al., 1959; Billaudelle et al., 1960). In addition, it was found to contain an agglutinogen

(Flosdorf and Kimball, 1940; Ehrich et al., 1942) and a hemagglutinin (Keogh et al., 1947; Masry, 1952). Thermostable (Maclennan, 1960; Sutherland, 1961) and thermolabile (Billaudelle et al., 1960; Banerjea and Munoz, 1962) toxins have also been described.

Bordetella pertussis produces a disease in infants and children characterized by tracheobronchitis and toxemia. The condition is manifested clinically in 3 stages. The first stage is a catarrhal tracheitis which lasts about 2 weeks and is characterized by a mild cough. This progresses in severity to a paroxysmal stage characterized by rapid consecutive coughs and a deep inspiratory whoop. This stage lasts about 2 weeks. During convalescence the paroxysms gradually decrease in severity and frequency. Pneumonia and otitis media are frequent complications.

The organism is present in large numbers in the respiratory tract during the catarrhal stage. In histological sections they are seen massed between the cilia of the tracheal and bronchial epithelial cells. The organism is seldom isolated after the fourth week of the disease or from normal individuals. In the latter part of the paroxysmal stage and during convalescence specific antibodies appear in the blood.

The lethal toxin is thought to assist in producing the generalized symptoms observed in pertussis. Individuals immunized with a toxoid develop clinical localized pertussis when exposed, but do not develop the generalized symptoms.

It is speculated that the histamine sensitizing factor and the hemagglutinin may also play roles in the pathogenesis of the disease.

Bordetella parapertussis was first isolated from a case of whooping cough by Bradford and Slavin in 1937. They reported that 8 of 160 isolates suspected of being Bordetella pertussis were Bordetella parapertussis. The disease produced by this organism is generally milder than that produced by Bordetella pertussis. Lacey (Lautrop and Lacey, 1960) reported that Bordetella pertussis was responsible for 95 percent of the cases of whooping cough in London, England, while Bordetella parapertussis was responsible for 5 percent and Bordetella bronchiseptica was responsible for 0.1 percent. Lautrop (Lautrop and Lacey, 1960) stated that in Denmark 20 to 25 percent of the cases were due to Bordetella parapertussis. Bordetella pertussis and Bordetella parapertussis are not known to cause natural infections in any species other than man.

MATERIALS AND METHODS

Culture Media

Isolation of Bordetella bronchiseptica from porcine nasal exudate is frequently difficult due to the presence of faster growing organisms. During the initial stages of this work this organism was found to grow readily on MacConkey's agar[1]. Most bacteria other than Bordetella bronchiseptica found in porcine nasal exudate do not grow on this medium. It was also found that addition of 1 percent dextrose to MacConkey's agar increased its selectivity. This medium is designated modified MacConkey's agar in this thesis.

Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. were grown on 5 percent bovine or equine blood agar. The base used in making the bovine blood agar was composed of the following ingredients:

Beef extract[1]	3.0 Grams
Proteose peptone #3[1]	7.5 Grams
Sodium chloride	5.0 Grams
Tryptose[1]	7.5 Grams
Agar	15.0 Grams
Distilled H_2O	1000.0 ml.

The base used in the equine blood agar was tryptose blood agar base[1]. Growth of Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. on these 2 media appeared to be equally satisfactory.

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[1]Difco Laboratories, Inc., Detroit, Michigan.

Broth media used for propagation of Bordetella bronchiseptica and Pasteurella multocida were tryptose phosphate broth[1] and a similar broth composed of the following ingredients:

Tryptose[1]	3.5 Grams
Proteose peptone #3[1]	10.0 Grams
Dextrose	0.2 Grams
Sodium chloride	5.0 Grams
Disodium phosphate	2.5 Grams
Beef extract[1]	5.0 Grams
Distilled H_2O	1000.0 ml.

Growth of these organisms was good in both media. Other media employed in biochemical studies were reconstituted dehydrated commercial media[1].

Inocula for transmission and pathogenicity trials were prepared by inoculating 0.1 ml. amounts of 24-hour broth cultures of the organism into the yolk sacs of 5- to 7-day-old embryonating hens' eggs. In Trial IV, reconstituted lyophilized second-passage cultures were inoculated into the embryos instead of the broth cultures. The embryos died after about 36 hours of incubation at 37 C. Yolk-sac fluids were harvested, checked for purity on blood agar and used as inocula.

Collection of Specimens for Culture

Nasal exudate was routinely collected from live pigs in the following manner. One external naris of a well-restrained

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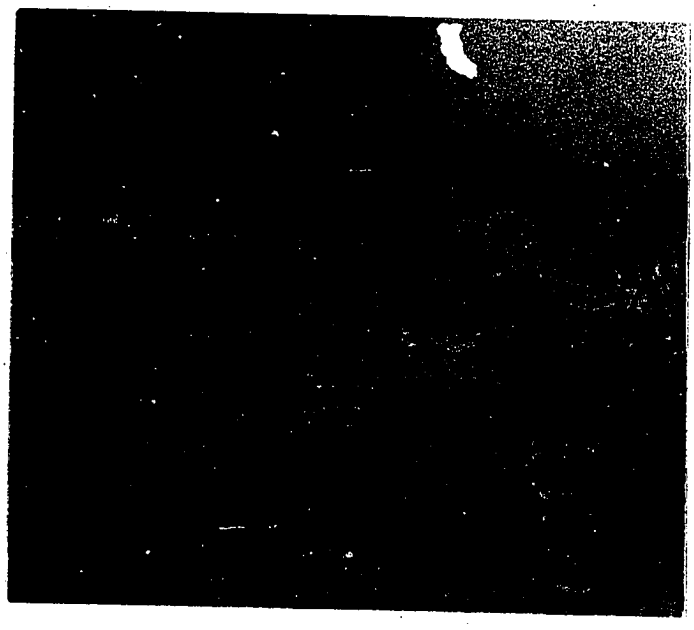
[1]Difco Laboratories, Inc., Detroit, Michigan.

pig was cleaned with cotton saturated with alcohol. A sterile cotton-tipped applicator was carefully inserted about one-third the length of the nasal cavity using a gentle twisting motion. Samples collected in this manner in Survey I were then suspended in 2 ml. of Dulbecco's phosphate broth (Dulbecco and Vogt, 1954) plus 10 percent calf serum within 2 hours after collection. In all other surveys and experimental work the nasal exudate samples were immediately streaked on media.

Nasal exudate was also collected from pigs examined at necropsy. The head was split longitudinally, the median septum was carefully removed and a sterile cotton-tipped applicator was inserted between the dorsal and ventral turbinates at the center of the nasal cavity. Figure 1 demonstrates the collection of nasal exudate from a pig at necropsy.

Tracheal exudate was aseptically collected with sterile cotton-tipped applicators from the lower trachea. Lung tissue was collected aseptically from the cardiac lobe. A piece approximately 0.5 $cm.^3$ was minced with scissors, ground with sterile 90-mesh alundum and suspended in broth. In Trials I and II this lung tissue was incubated at 37 C. for 24 hours and subcultured on modified MacConkey's agar. In Trial III the lung tissue suspension was subcultured without pre-incubation.

Figure 1. Collection of nasal exudate at necropsy



Isolation and Identification of Bordetella Bronchiseptica, Pasteurella Multocida and Hemophilus Spp.

Moderate-sized tan colonies that appeared on modified MacConkey's agar after 48 hours incubation were considered typical suspect colonies of Bordetella bronchiseptica. Positive isolates were identified as Gram negative rods producing urease in 2 to 12 hours, utilizing citrate in 24 to 48 hours, alkalinizing litmus milk and dextrose in 24 to 72 hours and not fermenting dextrose.

Typical colonies of Pasteurella multocida were picked from blood agar after 24 hours of incubation. Positive isolates were identified as Gram negative rods producing acid in dextrose but not in lactose, producing indole but not hydrogen sulfide and producing no change in litmus milk. Hemophilus spp. were isolated on blood agar by streaking a staphylococcus culture, known to supply growth factors, once across the blood agar. Typical satelliting colonies were picked and restreaked on blood agar with a staphylococcus streak line to confirm the satelliting and morphologic characteristics. No attempt was made to differentiate Hemophilus suis and Hemophilus parasuis.

Source of Bordetella Bronchiseptica Isolates

Isolates of Bordetella bronchiseptica used were from the nasal and tracheal exudate of various species of animals. These were collected from animals and specimens submitted to the Iowa Veterinary Diagnostic Laboratory and the Iowa Veterinary Medical Research Institute.

All isolates of Bordetella bronchiseptica used in transmission trials have been assigned a number and an abbreviation to indicate the species of origin. Abbreviations are "S" for swine, "D" for dog, "Rb" for rabbit, "Rt" for rat and "Ct" for cat.

Isolate S-1[1] was originally isolated from the nasal exudate of a pig in a surgically derived herd of swine that was experiencing an outbreak of rhinitis.

Isolate S-2 was a reisolate of S-1 from the nasal exudate of an experimentally infected 1-week-old pig. The pig had been infected intranasally with eighth-passage yolk-sac culture of Isolate S-1 on the fourth day of life.

Isolate S-3 was recovered from nasal exudate collected from a 4-month-old pig with definite nasal distortion and clinical signs of rhinitis. This animal had been on a therapeutic regime of sulfamethazine followed by sulfathiazole and was the only pig in the group to remain positive after the treatments.

Isolate S-4 was isolated from porcine nasal exudate submitted to the Iowa Veterinary Diagnostic Laboratory from a group of sneezing purebred pigs.

Isolate S-5 was recovered from another young pig with rhinitis and turbinate atrophy. It was submitted from a herd that had a clinical rhinitis problem. Isolate S-6 was

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[1]Appreciation is expressed to Dr. W. P. Switzer who recovered this isolate.

isolated from a pig submitted with a similar history.

Isolate D-1 was recovered from the nasal exudate of a young mongrel pup that had clinical signs of canine distemper. The organism was isolated from both the nasal and tracheal exudate of this dog. Histologic examination revealed inclusion bodies and other lesions of canine distemper.

Isolate Rb-1 was recovered from nasal exudate collected from a laboratory rabbit that had a chronic respiratory infection. The animal had been used to produce hyperimmune Bordetella bronchiseptica serum. Subsequent to isolating the organism from the nasal exudate, the animal was necropsied and lesions of rhinitis with moderate to severe turbinate atrophy were observed.

Isolate Rt-1[1] was recovered from the tracheal exudate of a wild rat trapped at a small town dump. Samples collected from several other rats from this dump were positive for Bordetella bronchiseptica.

Isolate Ct-1 was recovered from the tracheal exudate of a 2- to 3-month-old kitten. The kitten was thin, had encrusted exudate around the eyes and tracheal exudate.

Other isolates studied were obtained from the 8- to 10-week-old pigs sampled in Survey I, from swine submitted to the Iowa Veterinary Diagnostic Laboratory and from a collection belonging to Dr. W. P. Switzer. Certain of these isolates

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[1]Appreciation is expressed to Dr. W. P. Switzer who recovered this isolate.

were 8- to 10-years-old and had originally been identified as Alcaligenes fecalis-like organisms.

All isolates were stored on tryptose agar slants at room temperature. Isolates S-2, S-3, S-4, S-5, D-1, Rb-1, Rt-1 and Ct-1 were selected on initial isolation from primary 48-hour colonies on modified MacConkey's agar. After 48 hours of incubation in broth, a few drops of sterile calf serum were added and the cultures were lyophilized. The lyophilized isolates were stored at 4 C.

Biochemic, Metabolic and Morphologic Comparisons of Various Isolates of Bordetella Bronchiseptica

Comparison of the morphology of Bordetella bronchiseptica isolates was made using Gram's staining procedure and Leifson's flagellar stain (Leifson, 1960). Motility was studied using a brightfield illuminated light microscope with the condenser stopped down to increase the contrast.

Comparison of the biochemical activity of isolates of Bordetella bronchiseptica was made in Simmons citrate agar[1], litmus milk[1], urea agar[1], phenol red broth base[1] with 1 percent dextrose added, nitrate agar[1], gelatin[1], and S I M medium[1].

All media were inoculated with 24-hour broth cultures of fourth- to sixth-passage isolates of Bordetella bronchiseptica. They were incubated aerobically at 37 C. and observed at various intervals.

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[1]Difco Laboratories, Inc., Detroit, Michigan.

Indol formation was determined according to Kovac's method (Difco Laboratories, Inc., 1953). Determinations of the other activities were made according to the methods outlined in the Difco Manual (Difco Laboratories, Inc., 1953). Catalase production and cytochrome oxidase production were determined according to the procedures outlined by Wilson and Miles (1964) and Gaby and Free (1958) respectively.

Hemolysis was determined after 24 and 48 hours of incubation at 37 C. on 5 percent bovine, equine, sheep or rabbit blood agar.

Agglutination Tests

Serum collected from experimental animals was stored at -20 C. Prior to running plate or tube agglutination tests, samples of these sera were heated at 56 C. for 30 minutes and 1:10,000 merthiolate added as a preservative. Samples of serum collected from blood submitted to the Iowa Veterinary Diagnostic Laboratory for routine leptospirosis plate tests were also inactivated and preserved.

Antigen was prepared from 48-hour bovine blood agar cultures of the S-1 isolate of Bordetella bronchiseptica. In 1 instance a similar preparation was made of the S-4 isolate. The 48-hour-growth was scraped off and suspended in 0.85 percent sodium chloride solution.

The concentration of organisms in plate antigens was adjusted to contain approximately 2×10^{10} organisms per ml.

by the Wright method (Carpenter, 1956). This antigen was diluted 1:10 for use in the tube agglutination test.

It was found that live, 0.25 percent formaldehyde inactivated and 0.25 percent phenol inactivated preparations were comparable in their activity as plate antigens. Preparations boiled for 30 minutes or heated at 121 C. for 1 hour were considerably less active in the plate agglutination test.

A plate agglutination procedure was found to be useful in studies on Bordetella bronchiseptica. Equal quantities of various dilutions of antiserum and the plate antigen were mixed on a glass plate and incubated at room temperature for 8 minutes. Agglutination titers were graded 4+, 3+, 2+, 1+, and negative. The highest dilution of antiserum with a 2+ reaction was considered the titer of a given sample.

In the tube agglutination test, 0.5 ml. amounts of doubling dilutions of serum were made in 0.85 percent sodium chloride solution in 12 mm. x 100 mm. glass tubes. One-half ml. of the dilute antigen was then added to each tube. The serum dilution-antigen mixtures were incubated at room temperature for 1 hour on a platform shaker[1]. The tubes were secured at approximately a 45 degree angle and the shaker was set at about 90 strokes per minute. This was designed to closely simulate the procedure recommended for studies on pertussis by Lautrop and Lacey (1960). Replicate, unagitated

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[1]Eberbach and Son Co. Inc., Ann Arbor, Michigan.

tests incubated at room temperature for 4 hours were comparable in readibility and reactivity. Agglutination reactions were graded as 4+, 3+, 2+, 1+, and negative. The highest dilutions of antiserum with a 2+ reaction were considered the titer of a given sample.

Source of Experimental Animals

Swine used in this work were procured from an isolated, respiratory disease-free herd maintained at the Veterinary Medical Research Institute. This herd was founded with surgically derived stock in 1951 and all subsequent entries have been surgically derived. Continuous clinical observation of the animals raised in this herd has revealed no signs of respiratory disease. In addition, approximately 50 percent of the pigs raised each year are examined at necropsy for the presence of respiratory disease. Repeated sampling of nasal secretions from pigs raised in this herd has revealed no evidence of infection with Bordetella bronchiseptica or other bacteria known to produce respiratory disease.

Personnel caring for and managing this swine herd have no contact with other swine or other species of animals. Commonly recommended management, sanitation and breeding practices are strictly enforced. All rations and feeds are prepared on special order by local commercial sources. No antibiotics, arsenicals or other drug additives are used.

Incidence Surveys

Survey I

Purebred pigs from 87 of the more progressive swine herds in Iowa were sampled during September and October of 1962. Four of the best 8- to 10-week-old pigs were sampled in each herd. These samples were collected as described previously and suspended in broth. One drop of the suspended exudate was streaked on 5 percent horse blood agar and .2 to .3 ml. was streaked on modified MacConkey's agar. These cultures were examined for Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. The results of this survey were published (Ross et al., 1963b).

Survey II

Samples of nasal exudate were collected in the Iowa Veterinary Diagnostic Laboratory from pigs submitted from another group of 87 swine herds. These pigs were submitted to the laboratory for a variety of reasons in groups of 1 to 3 animals. They varied from 1 week to several months in age. In addition, samples of pneumonic lung tissue were collected from pigs with pneumonia. These specimens were cultured for Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. Observations were also made on the incidence of turbinate atrophy in these pigs.

Survey III

In the third survey, samples of nasal exudate from rhinitis suspects were submitted to the Iowa Veterinary Diagnostic Laboratory by veterinary practitioners. The ages of the sampled animals varied considerably. The number of animals sampled per herd varied from 3 to 50. Thirty-two herds were sampled. These specimens were cultured for Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp.

Survey IV

In this survey samples of nasal exudate were collected from pigs submitted to the Iowa Veterinary Diagnostic Laboratory as rhinitis suspects. The average age of these pigs was about 8 weeks. The sample size varied from 1 to 3 pigs. Twenty-eight herds were sampled. These specimens were cultured for Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. The incidence of turbinate atrophy was determined.

Transmission Trials

Trial I

Objectives The objectives of this trial were to determine the pathogenicity of an isolate of Bordetella bronchiseptica for 4-week-old pigs, to determine the prevalence of the organism in the respiratory tract and its

association with the lesions, and to determine the agglutinating antibody response of infected pigs to the organism.

Animals Thirty pigs were weaned at 3 weeks of age, randomized and divided into groups of 12 and 18 pigs. When they were 4 weeks of age, preinoculation nasal secretion samples were collected. All samples were negative for Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp.

Housing and feeding The 12 control pigs were housed in a central farrowing house. The 18 inoculated pigs were housed in groups of 9 in 2 isolation units. The temperature of the central building was maintained at 45 to 55 F. The temperature of the isolation units was maintained at this level for the first week. The windows were opened during the second week and the temperature frequently dropped below freezing. No bedding was used. A 16 percent grower ration was supplied in self-feeders. Water was supplied in troughs.

Inoculation Inoculum consisted of 1 ml. of sixth-passage yolk-sac culture of Bordetella bronchiseptica Isolate S-1. It was administered into the right nasal cavity with a syringe and a short piece of 2 mm. plastic tubing.

Necropsy and bacteriologic sampling Randomly selected pigs were necropsied in groups of 3 infected pigs and 2 control pigs at each of 6 intervals (1, 2, 3, 4, 6, and 8 weeks postinoculation). Samples of nasal exudate, tracheal exudate and lung tissue were collected as described previously. The lung tissue suspension was incubated at 37 C. for 24 hours

and subcultured on modified MacConkey's agar. The nasal exudate and tracheal exudate were immediately cultured on modified MacConkey's agar.

Each week after the necessary animals had been necropsied, samples of nasal exudate were collected from the remaining live pigs. These were cultured on modified MacConkey's agar.

Trial II

Objectives The objectives of Trial II were to determine the pathogenicity of an isolate of Bordetella bronchiseptica for 3-day-old pigs, to determine the prevalence of the organism in the respiratory tract and its association with lesions, and to determine the ability of this organism to infect adult swine.

Animals Three pregnant third-litter Landrace sows mated to a Hampshire boar were procured from the source herd approximately 1 week prior to parturition. These sows were allowed to farrow in isolation units. Two of the 3 sows did not deliver enough pigs, so extra newborn pigs of the same age were procured from the source herd and placed with the deficient litters.

Housing and feeding Each sow and litter was housed in an isolation unit which had approximately 50 square feet of floor space. Water and feed were supplied in troughs. Wood shavings were used for bedding.

Two ml. of injectible iron[1] was administered intramuscularly to each pig at 7 days of age. A starter feed was supplied at 2 weeks of age and the pigs were weaned at 4 weeks of age. Appropriate rations were supplied in self-feeders at various stages of growth.

Inoculation The sows were each given sixth-passage yolk-sac cultures of Bordetella bronchiseptica Isolate S-1 on 2 consecutive days prior to parturition. Two ml. of the inoculum was instilled in each nasal cavity with a syringe and a piece of 2 mm. plastic tubing. The pigs were given 0.5 ml. of the same inoculum at 3 days of age. They were inoculated by infusing the inoculum into the right nostril. The nostrils and mouth were then covered to induce inhalation.

Necropsy and bacteriologic sampling Pigs were randomly selected from each of the 3 litters so that each litter contributed equally to each necropsy period. Three animals were necropsied at each of 3 intervals (7 days, 14 days, and 21 days postinoculation) and 10 were necropsied at 5 weeks postinoculation. The remaining 11 pigs were necropsied at 5 months postinoculation.

Samples of nasal exudate, tracheal exudate and lung tissue were collected from all pigs at necropsy as previously described. The lung tissue suspensions were incubated at 37 C. for 24 hours, then subcultured on modified MacConkey's

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[1]Armidexan, Armour Veterinary Laboratories, Kankakee, Illinois.

agar. Samples of liver, spleen, kidney and heart were similarly collected, ground and suspended in broth. These were immediately subcultured on the modified MacConkey's agar.

Samples of nasal exudate were collected at weekly intervals during the first 3 weeks postinoculation. In addition, similar samples were collected from 11 pigs at 5 weeks, 12 weeks and 16 weeks postinoculation and at necropsy at 20 weeks postinoculation.

Trial III

Objective In Trial III the pathogenicity of 8 isolates of Bordetella bronchiseptica for baby pigs was compared under relatively standardized conditions.

Animals Eight groups of 4 purebred Yorkshire pigs were procured from the source herd at 1 day of age. These pigs had all nursed their dams several times. It was impossible to randomize these because the dates of birth varied over a several month period and it was necessary that all of the pigs be inoculated at the same age. In most instances, the animals inoculated with a given isolate were littermates. A few pigs died because of diarrhea. These were replaced with 1-day-old pigs from other litters.

Housing and feeding The procedure for box-rearing pigs outlined by Switzer et al. (1963) was used. Isolation units were set up with 4 cardboard egg cases in each unit. The temperature in each unit was set at 75 F. for the first

few days, then gradually dropped to about 50 F. Heat bulbs were suspended above the pigs to raise the temperature to a comfortable level. A commercial sow's milk replacer[1] was fed 4 times a day.

At about 10 days of age the pigs were removed from the boxes and placed on the unit floor. With some groups, wood shavings were kept in 1 corner of the unit, but in later groups the shavings were not used. A heat lamp was suspended in 1 corner to provide extra warmth.

Water was supplied in automatic waterers and an 18 percent protein starter was supplied in self-feeders. At 3-1/2 weeks of age the pigs were weaned. Personnel caring for these animals wore rubber rain suits and showered each time when entering and when leaving a unit.

Inoculation At 3 days of age each pig was given 0.25 ml. of third-passage yolk-sac culture of the appropriate Bordetella bronchiseptica isolate in each nostril.

Necropsy and bacteriologic sampling The pigs were necropsied at 4 weeks postinoculation. Samples of nasal exudate, tracheal exudate and lung tissue were collected and cultured on modified MacConkey's agar. The nasal exudate was also streaked on blood agar. The lung tissue suspensions were subcultured immediately on modified MacConkey's agar. The liver, spleen, 1 kidney and the heart of each pig were seared

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[1]S.P.F.-lac, Borden Company, 350 Madison Avenue, New York, New York.

with a hot spatula, incised and material collected on a 2 mm. loop was cultured on modified MacConkey's agar.

RESULTS

Development of a Selective Medium For Isolation of Bordetella Bronchiseptica

In the surveys and transmission trials reported in this work, MacConkey's agar modified by the addition of 1 percent dextrose was found to be a very useful selective medium for isolation of Bordetella bronchiseptica. This medium inhibited the growth of common organisms found in the porcine nasal cavity such as alpha hemolytic streptococci, Pasteurella multocida and Staphylococcus spp. However, Escherichia coli and certain other organisms when present in large numbers did grow on this medium and produced an acid medium which inhibited the growth of Bordetella bronchiseptica.

Bordetella bronchiseptica colonies developing on modified MacConkey's agar appeared as opalescent pin-point colonies after 24 hours of incubation. These colonies enlarged rapidly during the next 24 hours of incubation. Isolated colonies averaged 2 to 3 mm. in diameter, while densely crowded colonies were considerably smaller after 48 hours of incubation. These colonies were translucent, but had a distinct tan periphery with a darker center. Forty-eight-hour colonies of Bordetella bronchiseptica on modified MacConkey's agar were circular in outline and low convex in elevation. The surface was usually smooth, although primary colonies in a few instances were quite rough with irregular contours. No attempt was made to identify intermediate forms.

Colonies developing on blood agar were more opaque, dome-shaped and smaller in size than those developing on modified MacConkey's agar. These 48-hour colonies on blood agar resembled a bisected pearl. Figure 2 demonstrates the 48-hour growth of Bordetella bronchiseptica Isolate S-1 on modified MacConkey's agar. Figure 3 shows the same isolate on 5 percent bovine blood agar.

Incidence Surveys

Survey I

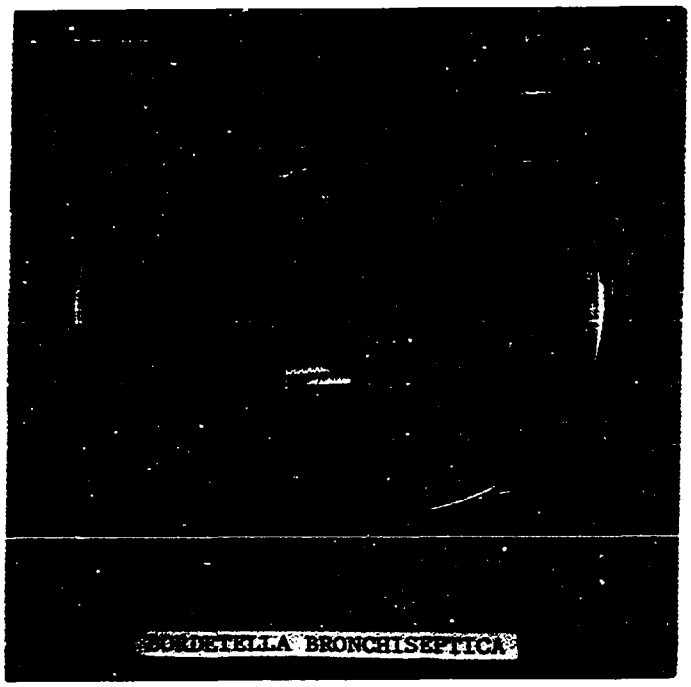
Culture of samples of nasal exudate collected from four 8- to 10-week-old pigs from each of 87 Iowa swine herds revealed that the herd incidence of Bordetella bronchiseptica, Hemophilus spp. and Pasteurella multocida was 54 percent (47/87), 48 percent (42/87) and 6 percent (5/87) respectively. Twenty-eight percent (24/87) of these herds were negative for all 3 species. Additional information on the incidence of these 3 bacteria is presented in Table 1.

Survey II

Survey II consisted of cultural examination of samples of nasal exudate collected at necropsy from a second group of 87 herds (124 pigs) of Iowa swine. The herd incidence of Bordetella bronchiseptica, Hemophilus spp. and Pasteurella multocida was 38 percent (33/87), 23 percent (20/87) and 9 percent (8/87) respectively. Forty-five percent (39/87) of

Figure 2. Forty-eight-hour growth of Bordetella bronchiseptica Isolate S-1 on modified MacConkey's agar

Figure 3. Forty-eight-hour growth of Bordetella bronchiseptica Isolate S-1 on 5 percent bovine blood agar


BORDETELLA BRONCHISEPTICA


BORDETELLA BRONCHISEPTICA

Table 1. Incidence of infections of Bordetella bronchiseptica, Pasteurella multocida, and Hemophilus spp. in Surveys I and II

Organism	Survey I (87 herds)		Survey II (87 herds)	
	No. herds	Percent herds	No. herds	Percent herds
Bordetella bronchiseptica	21	24	25	29
Bordetella bronchiseptica and Hemophilus spp.	22	25	4	5
Bordetella bronchiseptica and Pasteurella multocida	1	1	4	5
Bordetella bronchiseptica Pasteurella multocida and Hemophilus spp.	3	3	0	0
Pasteurella multocida	0	0	0	0
Pasteurella multocida and Hemophilus spp.	1	1	0	0
Hemophilus spp.	15	17	16	18
No organisms isolated	24	28	39	45

the herds were negative for all 3 bacteria. More detailed information on the incidence of these 3 organisms in Survey II is presented in Table 1 along with comparable data from Survey I.

Turbinate atrophy was observed in at least 1 pig from each of 24 of the 87 (28 percent) herds. On a pig basis, the incidence of turbinate atrophy was 26 percent (32/124). The

incidence of turbinate atrophy in relation to the bacteria isolated is presented in Table 2.

Table 2. Correlation of turbinate atrophy and isolation of Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. in Survey II

Bacteria isolated[a]	No. pigs positive	No. pigs w/turbinate atrophy	Percent pigs w/turbinate atrophy
Bordetella bronchiseptica	34	10	29
Hemophilus spp.	16	5	31
Pasteurella multocida	4	2	50
Bordetella bronchiseptica and Pasteurella multocida	3	1	33
Bordetella bronchiseptica and Hemophilus spp.	4	0	0

[a]Combinations of the 3 bacteria other than those presented were not encountered in this survey on a pig basis.

Forty-three percent (53/124) of the pigs necropsied in Survey II had lesions of pneumonia. On a herd basis the incidence of pneumonia was 44 percent (38/87). Thirty-two percent (17/53) of the pigs with pneumonia had lesions of turbinate atrophy. Table 3 contains information on the bacteria isolated from the nasal cavities and lungs of 35 of the pigs with pneumonia.

Table 3. Incidence of Bordetella bronchiseptica, Hemophilus spp. and Pasteurella multocida in the nasal cavities and lungs of 35 pigs with pneumonia in Survey II

Bacteria isolated	No. of pigs positive Nasal cavity	Lungs
Bordetella bronchiseptica[a]	10	2
Hemophilus spp.	3	2
Pasteurella multocida[b]	5	10

[a]Bordetella bronchiseptica was isolated from both the pneumonic lungs and nasal cavity of one pig.

[b]Pasteurella multocida was isolated from both the pneumonic lungs and nasal cavities of two pigs.

Survey III

Culture of samples of nasal exudate submitted by veterinary practitioners from pigs in 32 herds suspected of having rhinitis revealed that 56 percent (18/32) had Bordetella bronchiseptica and 16 percent (5/32) had Hemophilus spp. Pasteurella multocida was not detected.

Survey IV

Culture of samples of nasal exudate collected from pigs from each of 28 Iowa swine herds suspected of having rhinitis revealed that the herd incidence of Bordetella bronchiseptica, Pasteurella multocida and Hemophilus spp. was 68 percent (19/28), 11 percent (3/28), and 11 percent (3/28) respectively. Seventy-one percent (20/28) of these herds had pigs with

turbinate atrophy. In 68 percent (13/19) of the herds with Bordetella bronchiseptica, gross turbinate atrophy was observed.

Transmission Trials

Trial I

Eighteen 4-week-old pigs inoculated with Bordetella bronchiseptica Isolate S-1 sneezed and coughed intermittently from the fifth day to the fourth week postinoculation. Sneezing and coughing were rarely observed from the fourth week to the termination of the trial. No evidence of respiratory disease was observed in the 12 control pigs at any time.

Three pigs necropsied 1 week after inoculation had a slight catarrhal rhinitis. Ten of 15 pigs necropsied from the second week to the eighth week postinoculation had lesions of slight to moderate turbinate atrophy. The 12 control pigs had normal turbinates. Detailed information on the necropsy findings in Trial I is presented in Table 4.

In comparison with the turbinates of control animals, the turbinates of inoculated pigs were ischemic, covered with catarrhal exudate, softened because of loss of bony core and atrophic. The ventral turbinates were invariably the most severely affected. The ventral scroll of the ventral turbinates was always more atrophic than the dorsal scroll. The ethmoid turbinates were frequently slightly atrophic while atrophy of the dorsal turbinates was infrequent. The degree

Table 4. Necropsy, bacteriologic and serologic findings in 18 pigs inoculated intranasally at 4 weeks of age with Bordetella bronchiseptica Isolate S-1

Necropsy interval		Turbinate atrophy	Recovery of Bordetella bronchiseptica			Agglutination titer	
			Nasal Cavity	Trachea	Lungs	Plate	Tube
One week	Pig one	none	+	+	+	0	0
	Pig two	none	+	+	-	0	0
	Pig three	none	+	+	-	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Two weeks	Pig one	none	+	+	-	0	0
	Pig two	slight bilateral	+	+	-	0	0
	Pig three	none	+	+	+	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Three weeks	Pig one	slight unilateral	+	+	-	0	0
	Pig two	moderate bilateral	+	+	+	0	0
	Pig three	moderate unilateral	+	+	-	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0

Table 4. (continued)

Necropsy interval		Turbinate atrophy	Recovery of Bordetella bronchiseptica			Agglutination titer	
			Nasal Cavity	Trachea	Lungs	Plate	Tube
Four weeks	Pig one	none	+	+[a]	-	0	0
	Pig two	moderate bilateral	+	+[a]	-	0	0
	Pig three	slight unilateral	+	+[a]	-	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Six weeks	Pig one	none	+	+[a]	-	0	0
	Pig two	moderate bilateral	+	-	+	0	2[b]
	Pig three	slight unilateral	-	-	-	0	4
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Eight weeks	Pig one	slight bilateral	+	-	-	16	64
	Pig two	slight unilateral	+	-	-	16	64
	Pig three	none	+	-	-	16	64
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0

[a]Colonies reduced in number.

[b]Titer as determined by highest serum dilution with a 2+ reaction or higher.

of turbinate atrophy was estimated by comparing the size and relative space occupied by the turbinates of each inoculated pig with those of comparable control pigs. Figures 4, 5 and 6 demonstrate lateral views of turbinates from pigs with slight atrophy, moderate atrophy and no atrophy respectively.

Pneumonia was not observed in any of the 18 inoculated pigs. However, increased tracheal secretions were frequently observed in these pigs. The 12 control pigs had normal lungs and tracheas.

Bordetella bronchiseptica was recovered in heavy growth from the nasal exudate of all but 1 of the inoculated pigs at necropsy. In addition, the organism was recovered in heavy growth from the tracheal exudate of all pigs necropsied from the first to the third week postinoculation. The organism disappeared from the trachea during the fourth to eighth week postinoculation. Bordetella bronchiseptica was recovered from the grossly normal lungs of 4 pigs. Samples of nasal secretions, tracheal secretions and lung tissue collected from the control pigs were negative for Bordetella bronchiseptica. The bacteriologic findings on these pigs are presented in Table 4.

Plate and tube agglutination tests were conducted with serum from blood samples collected at necropsy from all inoculated and uninoculated pigs. Two pigs necropsied at 6 weeks postinoculation had titers of 1:2 and 1:4 and 3 pigs necropsied at 8 weeks postinoculation had titers of 1:64 in the tube agglutination test. The results of these tests are

Figure 4. Mild turbinate atrophy 2 weeks postinoculation in a 6-week-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 5. Moderate turbinate atrophy 3 weeks postinoculation in a 7-week-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1


56B

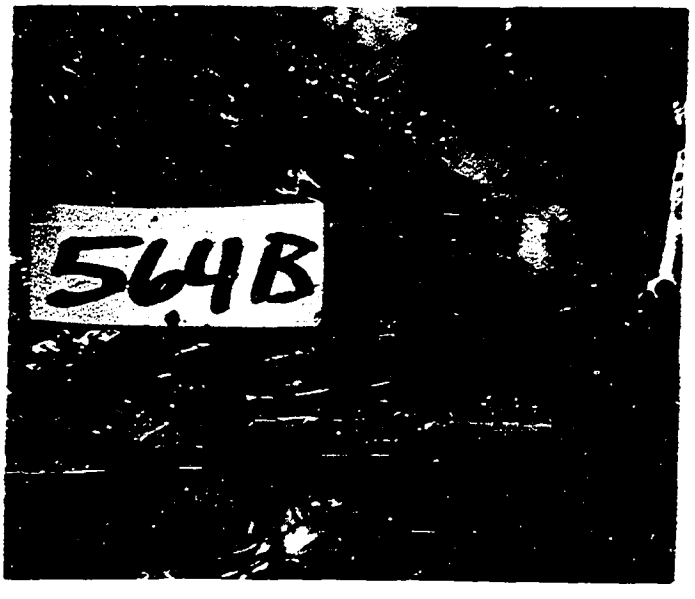

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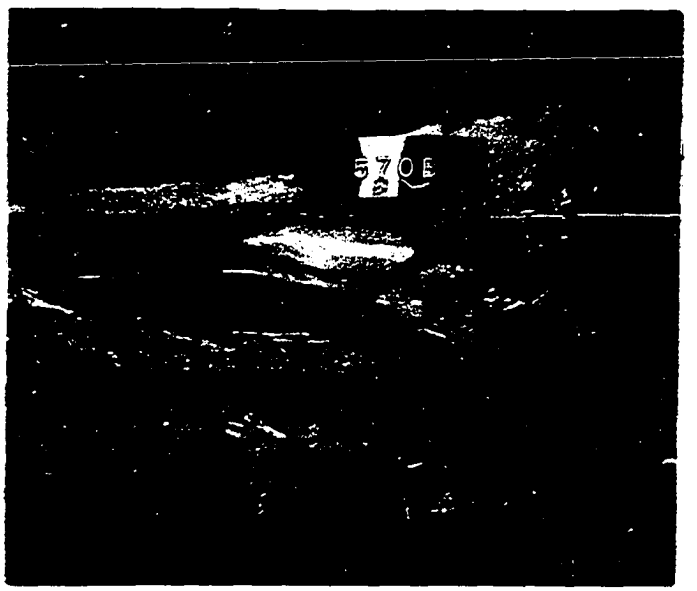
Figure 6. Normal turbinates of an uninoculated 8-week-old pig

Observe the normal redness of the turbinate mucosa in comparison to the relatively ischemic turbinates in Figures 3 and 4.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also recorded in Table 4.

Samples of nasal exudate collected from pigs remaining each week after the necessary animals had been necropsied were uniformly positive for Bordetella bronchiseptica from the second week postinoculation to the sixth week postinoculation. Similar samples collected from the control pigs were negative. Results of the bacteriologic examinations of these samples are presented in Table 5.

Table 5. Number of live pigs positive on nasal exudate culture for Bordetella bronchiseptica at various intervals postinoculation

Animals	Weeks postinoculation				
	1	2	3	4	6
No. of inoculated pigs positive	0	12	9	6	3
No. of inoculated pigs sampled	15	12	9	6	3
No. of control pigs positive	0	0	0	0	0
No. of control pigs sampled	10	8	6	4	2

Trial II

Three pregnant sows inoculated on 2 consecutive days with Bordetella bronchiseptica Isolate S-1 showed no signs of respiratory disease. Samples of nasal exudate collected from these sows 24 hours, 2 weeks, and 5-1/2 weeks after the second inoculation were negative for Bordetella bronchiseptica.

Thirty pigs nursing these sows and inoculated with Bordetella bronchiseptica Isolate S-1 at 3 days of age developed limited sneezing 5 to 10 days after inoculation. The sneezing was never marked and it subsided about 3 weeks postinoculation. Mild to moderate coughing was observed in a few pigs.

One pig necropsied 1 week after inoculation had slight turbinate atrophy and a catarrhal rhinitis. The other 2 inoculated pigs necropsied at this interval had a catarrhal rhinitis. Fifteen of 16 pigs necropsied from the second to the fifth week postinoculation had lesions varying from slight to severe turbinate atrophy. Eight control pigs necropsied at the various intervals had normal turbinates. Additional information on the necropsy findings in these pigs is presented in Table 6.

The turbinate atrophy was more pronounced in Trial II than in Trial I. However, the gross lesions were very similar in most respects to those observed in the older pigs. Figures 7, 8, 9, 10, 11, 12 and 13 demonstrate turbinates with various degrees of atrophy. Figure 14 demonstrates normal turbinates in a 3-1/2-week-old pig.

Small areas of pneumonia were found in the lungs of 1 pig necropsied at 3 weeks postinoculation and in 1 pig necropsied at 5 weeks postinoculation. Increased tracheal secretions were frequently observed in the inoculated pigs. Control pigs

Table 6. Necropsy, bacteriologic and serologic findings in 30 pigs inoculated intranasally at 3 days of age with Bordetella bronchiseptica Isolate S-1

Necropsy interval		Turbinate atrophy	Recovery of Bordetella bronchiseptica			Agglutination titer	
			Nasal cavity	Trachea	Lung	Plate	Tube
One week	Pig one	mild bilateral	+	+	+	0	0
	Pig two	none	+	+	-	0	0
	Pig three	none	+	+	-	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Two weeks	Pig one	moderate bilateral	+	+	-	0	0
	Pig two	moderate bilateral	+	+	-	0	0
	Pig three	moderate bilateral	+	+	+	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Three weeks	Pig one	moderate bilateral	+	+	-	0	0
	Pig two	moderate bilateral	+	+	-	0	0
	Pig three	moderate bilateral	+	+	+	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Five weeks	Pig one	severe bilateral	+	+	-	0	0
	Pig two	mild bilateral	+	+	-	0	0
	Pig three	severe bilateral	+	+	-	0	0
	Pig four	severe bilateral	+	+	+	0	0
	Pig five	severe bilateral	+	+	-	0	0

Table 6. (continued)

Necropsy interval		Turbinate atrophy	Recovery of *Bordetella bronchiseptica*			Agglutination titer	
			Nasal cavity	Trachea	Lung	Plate	Tube
Five weeks (continued)	Pig six	severe bilateral	+	+	-	0	0
	Pig seven	mild bilateral	+	+	-	0	0
	Pig eight	severe bilateral	+	+	-	0	0
	Pig nine	none	+	+	-	0	0
	Pig ten	severe bilateral	+	+	+	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
Twenty weeks	Pig one	none (distorted)[a]	-	-	-	0	0
	Pig two	none	-	-	-	0	0
	Pig three	none (distorted)[a]	+	-	-	4	8
	Pig four	none (distorted)[a]	+	-	-	0	4
	Pig five	none (distorted)[a]	-	-	-	4	8
	Pig six	none (distorted)[a]	-	-	-	0	2
	Pig seven	none (distorted)[a]	-	-	-	2	8
	Pig eight	none (distorted)[a]	-	-	-	0	4
	Pig nine	none	-	-	-	0	2
	Pig ten	none	-	-	-	0	8
	Pig eleven	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0
	Control pig	none	-	-	-	0	0

[a]Turbinates ischemic and distorted, but occupying approximately the normal dorso-ventral and medial-lateral area.

Figure 7. Catarrhal rhinitis 1 week postinoculation in a 10-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 8. Mild turbinate atrophy 2 weeks postinoculation in a 17-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

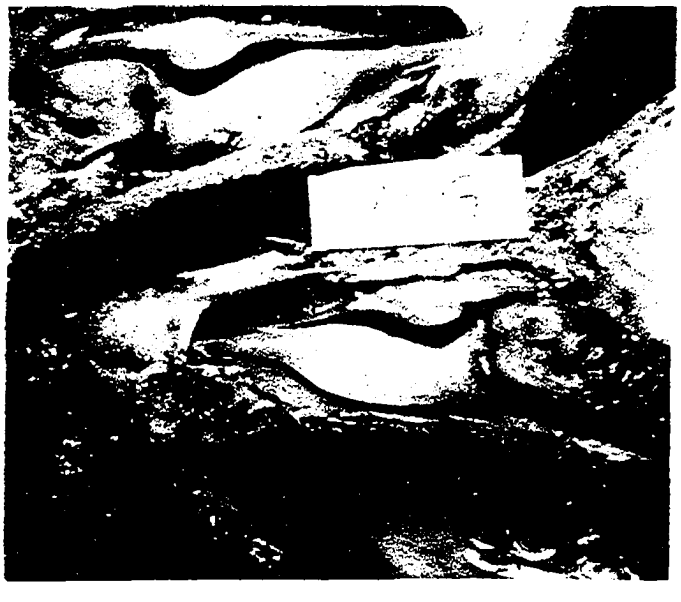

Figure 9. Moderate turbinate atrophy 3 weeks postinoculation in a 24-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 10. Moderate turbinate atrophy 3 weeks postinoculation in a 24-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

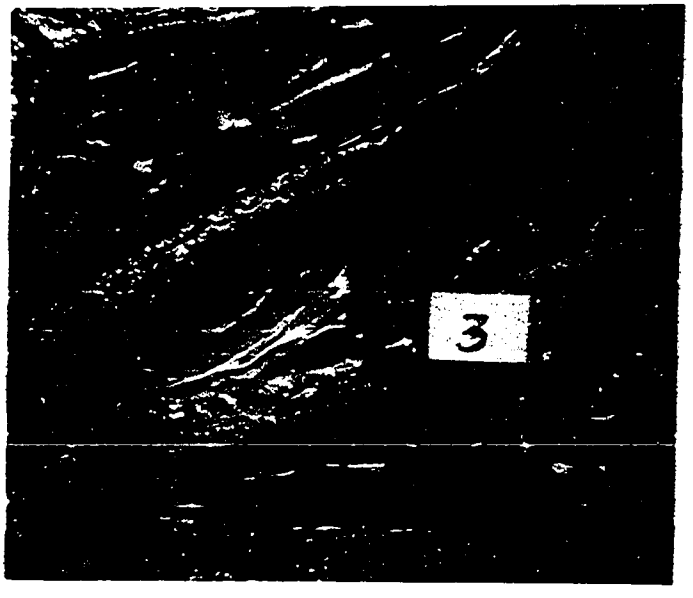
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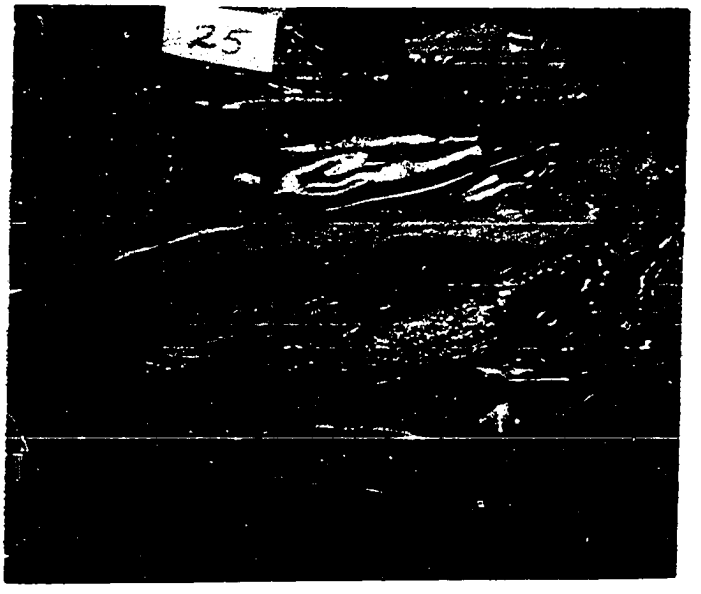
Figure 11. Severe turbinate atrophy 5 weeks postinoculation in a 38-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 12. Severe turbinate atrophy 5 weeks postinoculation in a 38-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 13. Severe turbinate atrophy 5 weeks postinoculation in a 38-day-old pig inoculated intranasally with Bordetella bronchiseptica Isolate S-1

Figure 14. Normal turbinates of an uninoculated 24-day-old pig

Observe the normal redness of the turbinate mucosa in comparison to the relatively ischemic turbinates in Figures 7 through 13.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necropsied at the various intervals had normal lungs and tracheas.

Examination of the turbinates of 11 pigs necropsied at 20 weeks postinoculation revealed that their ventral turbinates had normal dorso-ventral and medial-lateral dimensions and occupied the normal amount of space in the nasal cavities. However, some of these turbinates were ischemic in comparison with those from normal 20-week-old pigs and were distorted with longitudinal grooves and folds. Detailed information is presented in Table 6. Figures 15 and 16 demonstrate turbinates from a Bordetella bronchiseptica infected 20-week-old pig with distorted turbinates and from a normal 20-week-old pig respectively.

Bordetella bronchiseptica was isolated in heavy growth from samples of nasal and tracheal exudate collected from all pigs necropsied at 1 through 5 weeks postinoculation. It was also isolated from the lungs of the 2 pigs with pneumonia. In addition, 1 pig at 1 week postinoculation, 1 pig at 2 weeks postinoculation and 1 pig at 3 weeks postinoculation had the organism in grossly normal lungs. Two of the 11 animals necropsied at 20 weeks postinoculation had small numbers of Bordetella bronchiseptica in their nasal cavities. None of these 11 pigs had the organism in their lungs or tracheas. The results of the bacteriologic examinations on all pigs in Trial II are recorded in Table 6.

Plate and tube agglutination tests revealed that none of

Figure 15. Distorted ventral turbinate of a 20-week-old pig inoculated intranasally at 3 days of age with Bordetella bronchiseptica Isolate S-1

Figure 16. Normal turbinates of an uninoculated 20-week-old pig

the pigs necropsied from 1 through 5 weeks postinoculation developed agglutinating antibodies. Eight of 11 pigs necropsied at 20 weeks postinoculation had tube agglutination titers ranging from 1:2 to 1:8. Sera from the 2 control pigs necropsied at 20 weeks of age and 4 other uninoculated 20-week-old pigs were negative. These results are presented in Table 6.

Preinoculation sera collected from the 3 sows had tube agglutination titers of 1:8. Sera collected from these sows 4 weeks postinoculation also had titers of 1:8. Sera were collected from blood samples submitted from 15 different swine herds for routine leptospirosis plate agglutination tests. The animals ranged in age from 6 months to 4 years. Tube agglutination tests revealed that 24 of 35 animals from these herds had tube agglutination titers to Bordetella bronchiseptica Isolate S-1 ranging from 1:2 to 1:16.

The results of bacteriologic examinations of samples of nasal exudate collected from the remaining live pigs after each necropsy interval are recorded in Table 7.

Trial III

Isolate S-2 (swine origin) Pigs inoculated with Bordetella bronchiseptica Isolate S-2 coughed for a limited period during the second week postinoculation. Sneezing was never prevalent. One pig died 2 weeks after inoculation. Necropsy of this pig revealed moderate turbinate atrophy and

Table 7. Number of live pigs positive on nasal exudate culture for Bordetella bronchiseptica at various intervals postinoculation

Animals	Weeks postinoculation					
	1	2	5	12	16	20[a]
No. of inoculated pigs positive	20	21	11	11[b]	4[b]	2[b]
No. of inoculated pigs sampled	28	25	11	11	11	11

[a]Samples collected at postmortem.

[b]Number of colonies markedly reduced.

extensive pneumonia of the apical and cardiac lobes of the lungs. Postmortem examination of the other 3 pigs at 4 weeks postinoculation revealed slight to moderate bilateral turbinate atrophy and moderate involvement of the apical and cardiac lobes of the lungs with pneumonia.

Culture of nasal exudate from all 4 pigs on modified MacConkey's agar and 5 percent equine blood agar revealed heavy growth of Bordetella bronchiseptica. No other significant organisms were isolated. Culture of tracheal exudate from these animals revealed heavy growth of the organism. It was also recovered from the pneumonic lungs of all 4 animals.

Isolate S-3 (swine origin) Two pigs inoculated with Bordetella bronchiseptica Isolate S-3 started coughing about 10 days postinoculation and coughed with increasing frequency and severity until they were necropsied at 4 weeks

postinoculation. The other 2 pigs were not observed to cough and sneezing was never observed in any of the pigs.

Necropsy revealed that 3 pigs had moderate bilateral turbinate atrophy and 1 pig had slight to moderate bilateral turbinate atrophy. Extensive pneumonia was observed in the apical, cardiac and diaphragmatic lobes of the lungs of 2 pigs. One pig had small areas of pneumonia in the apical and cardiac lobes while the fourth pig had normal lungs.

Culture of nasal exudate from all 4 pigs on modified MacConkey's agar and 5 percent equine blood agar revealed heavy growth of Bordetella bronchiseptica. No other significant organisms were isolated. Bordetella bronchiseptica was isolated in heavy growth from the tracheal exudate of these pigs. It was also isolated from the pneumonic lungs of 3 pigs, but not from the normal lungs of the fourth pig.

Isolate S-4 (swine origin) The pigs inoculated with Bordetella bronchiseptica Isolate S-4 coughed mildly about 10 days after inoculation. No evidence of respiratory disease was observed throughout the rest of this trial.

Necropsy of these pigs revealed moderate to severe turbinate atrophy in 1 pig, slight to moderate bilateral turbinate atrophy in a second pig, slight to moderate unilateral turbinate atrophy in a third pig, and normal turbinates in the fourth pig. The lungs were normal in all 4 pigs.

Culture of nasal exudate from these pigs on modified

MacConkey's agar and 5 percent equine blood agar revealed heavy growth of Bordetella bronchiseptica. No other significant organisms were isolated. Culture of tracheal exudate from these pigs revealed heavy growth of Bordetella bronchiseptica. Approximately 10 colonies of the organism grew from the normal lungs of 2 pigs. Culture of the lungs from the other 2 pigs did not yield the organism.

Isolate S-5 (swine origin) The pigs inoculated with Bordetella bronchiseptica Isolate S-5 coughed mildly for 2 or 3 days about 10 days postinoculation. Necropsy revealed slight to moderate bilateral turbinate atrophy in all 4 pigs. One pig had extensive lesions of pneumonia. Two pigs had small lesions of pneumonia in the right apical lobe and the fourth pig had normal lungs.

Bordetella bronchiseptica was isolated in heavy growth on modified MacConkey's agar and 5 percent equine blood agar from the nasal exudate of all 4 pigs. No other significant organisms were isolated. Culture of tracheal exudate from these pigs revealed heavy growth of Bordetella bronchiseptica. It was isolated from the pneumonic lungs of 3 pigs, but not from the normal lungs of the fourth pig.

Isolate D-1 (canine origin) Isolate D-1 produced no coughing or sneezing. Necropsy revealed normal lungs and turbinates in all 4 pigs.

Culture of nasal exudate from these animals on modified MacConkey's agar and 5 percent equine blood agar revealed

heavy growth of Bordetella bronchiseptica from only 1 pig. Culture of the nasal cavities of the other 3 pigs revealed relatively few colonies. One culture plate had 3 colonies, 1 had 10 colonies, while the third had 50 to 75 colonies. No other significant organisms were isolated. Bordetella bronchiseptica was isolated in limited numbers from 3 of the 4 tracheal exudate samples. The fourth sample was negative. The lungs of 3 of these pigs were negative for Bordetella bronchiseptica while the fourth lung was positive for the organism.

Isolate Rb-1 (rabbit origin) Inoculation of Bordetella bronchiseptica Isolate Rb-1 produced a limited amount of sneezing and coughing about 7 to 10 days postinoculation. This lasted for about 1 week. Postmortem examination of these pigs at 4 weeks postinoculation revealed slight turbinate atrophy in 3 pigs and normal turbinates in 1 pig. Two pigs had moderate lesions of pneumonia in the dependent portion of the apical and cardiac lobes of their lungs.

Culture of nasal exudate from these animals on modified MacConkey's agar and 5 percent equine blood agar revealed moderate numbers of colonies (50 to 100) of Bordetella bronchiseptica. No other significant organisms were isolated. Culture of tracheal exudate revealed heavy growth of the organism from all 4 animals. The lungs of 3 pigs were positive for Bordetella bronchiseptica and the lungs of the fourth pig were negative for the organism.

Isolate Ct-1 (cat origin) Pigs inoculated with Bordetella bronchiseptica Isolate Ct-1 started coughing 5 days after inoculation. This persisted for about 2 weeks. Sneezing was evident, but minimal. One pig died 12 days after inoculation. Necropsy of this pig revealed extensive lesions of pneumonia in the apical, cardiac and diaphragmatic lobes. Moderate turbinate atrophy was observed. Necropsy of the other 3 pigs at 4 weeks postinoculation revealed moderate turbinate atrophy in 1 pig and slight to moderate turbinate atrophy in the other 2 pigs. One pig had moderate lesions of pneumonia in the apical and cardiac lobes. A second pig had small areas of pneumonia in the cardiac lobes while the third pig had normal lungs.

Culture of nasal exudate from all 4 pigs on modified MacConkey's agar and 5 percent bovine blood agar revealed heavy growth of Bordetella bronchiseptica. No other significant organisms were isolated. Culture of tracheal exudate from these pigs revealed heavy growth of Bordetella bronchiseptica. The pneumonic lungs of 3 pigs were positive for Bordetella bronchiseptica. The organism was isolated in heavy growth from the normal lungs of 1 pig.

Isolate Rt-1 (rat origin) Inoculation of Bordetella bronchiseptica Isolate Rt-1 produced a limited amount of coughing about 7 days postinoculation. Sneezing was never observed and the coughing soon subsided. One pig died 2-1/2 weeks postinoculation. Necropsy revealed slight turbinate

atrophy and normal lungs. The pig was severely dehydrated as a result of a chronic catarrhal enteritis. Postmortem examination of the other 3 pigs at 4 weeks postinoculation revealed normal turbinates in all 3 animals. Two pigs had normal lungs and 1 pig had small areas of congestion in 1 apical lobe.

Culture of nasal exudate from the pig that died revealed heavy growth of Bordetella bronchiseptica. Culture of nasal exudate from the other 3 pigs revealed moderate numbers (50 to 100) of colonies of the organism. Culture of these samples on 5 percent bovine blood agar revealed no other significant organisms. Bordetella bronchiseptica was isolated in heavy growth from the tracheas of 2 pigs. The trachea of the third pig was negative. Culture of the lungs from these three pigs revealed heavy growth of the organism from 1 pig and 10 colonies from 1 pig. The lungs of the third pig were negative.

The gross lesions produced by the 8 isolates were quite similar. The main variation appeared to be in the severity of these lesions. The turbinates were ischemic, softened due to loss of bony core and atrophic. The ventral turbinates were most severely affected and atrophy of the ethmoid turbinates was observed in most pigs. The dorsal turbinates were also frequently mildly atrophic. The involvement was bilateral in almost all cases.

Catarrhal exudate was observed infrequently on the nasal mucosa of these pigs. However, it was frequently observed in

tracheas. Pneumonia was quite extensive in some pigs. Lesions of subacute to acute pneumonia were reddish-brown while the chronic lesions were gray in color. These chronic lesions were quite firm and fibrous. Small areas of hemorrhage were observed in cross sections of these pneumonic areas. The results of this trial are summarized in Table 8.

Turbinate atrophy produced by Isolates S-3 (swine origin) and S-4 (swine origin) are demonstrated in Figures 17 and 18 respectively. Turbinate atrophy produced by Isolate Ct-1 (cat origin) is demonstrated in Figures 19 and 20. Turbinate atrophy produced by Isolate Rb-1 (rabbit origin) is demonstrated in Figure 21. Normal turbinates from a pig inoculated with Isolate D-1 (canine origin) are demonstrated in Figure 22. The acute and chronic lesions of pneumonia produced by Bordetella bronchiseptica are demonstrated in Figures 23 and 24 respectively.

Plate and tube agglutination tests were run on sera collected from all of the pigs in Trial III. In many instances these sera did not react or reacted very weakly with the S-1 antigen. The results of this work are recorded in Table 9.

Biochemic, Metabolic and Morphologic Comparisons of Various Isolates of Bordetella Bronchiseptica

The 8 isolates of Bordetella bronchiseptica used in Trial III were compared morphologically and by biochemic and metabolic tests. All 8 isolates (S-2, S-3, S-4, S-5, D-1,

Table 8. Incidence of turbinate atrophy, pneumonia and recovery of Bordetella bronchiseptica in pigs infected with eight different isolates of the organism

	Isolate							
	S-2	S-3	S-4	S-5	D-1	Rb-1	Ct-1	Rt-1
Turbinate atrophy	4/4[a]	4/4	3/4	4/4	0/4	3/4	4/4	1/4
Pneumonia	4/4	3/4	0/4	3/4	0/4	2/4	3/4	2/4
Organism isolated-nasal exudate	4/4	4/4	4/4	4/4	4/4[b]	4/4[b]	4/4	4/4[b]
Organism isolated-tracheal exudate	4/4	4/4	4/4	4/4	3/4[b]	4/4	4/4	3/4
Organism isolated-lungs	4/4	3/4	2/4	3/4	1/4	3/4	3/4	2/4

[a]Numerator equals number of pigs positive. Denominator equals number of pigs in group.

[b]Colonies reduced in number.

Figure 17. Moderate turbinate atrophy 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate S-3 (swine origin)

Figure 18. Moderate turbinate atrophy 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate S-4 (swine origin)

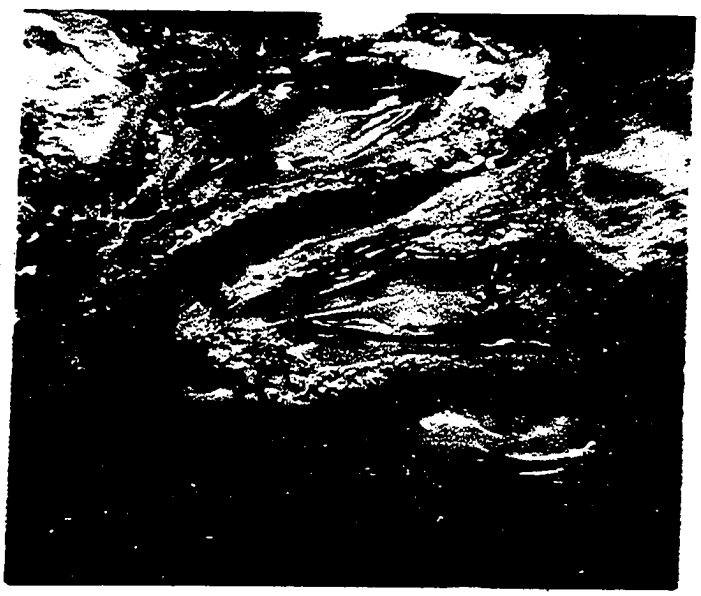
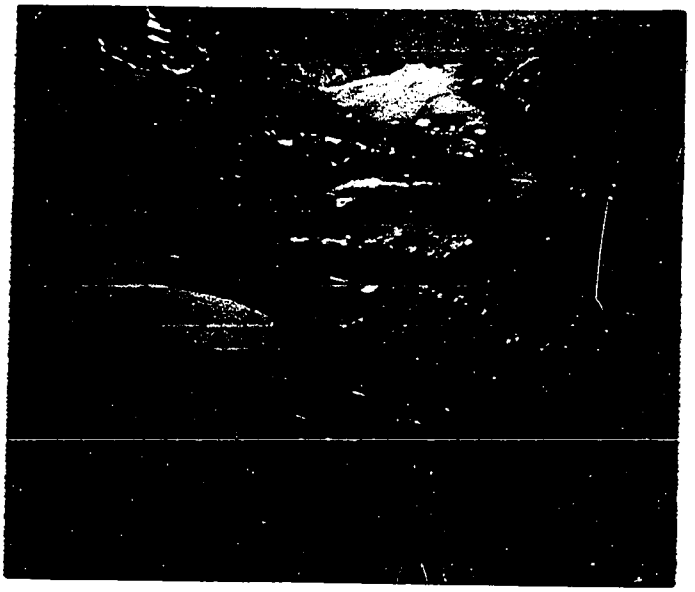


Figure 19. Moderate turbinate atrophy 12 days postinoculation in a 15-day-old pig inoculated with Bordetella bronchiseptica Isolate Ct-1 (cat origin)

Figure 20. Moderate turbinate atrophy 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate Ct-1 (cat origin)



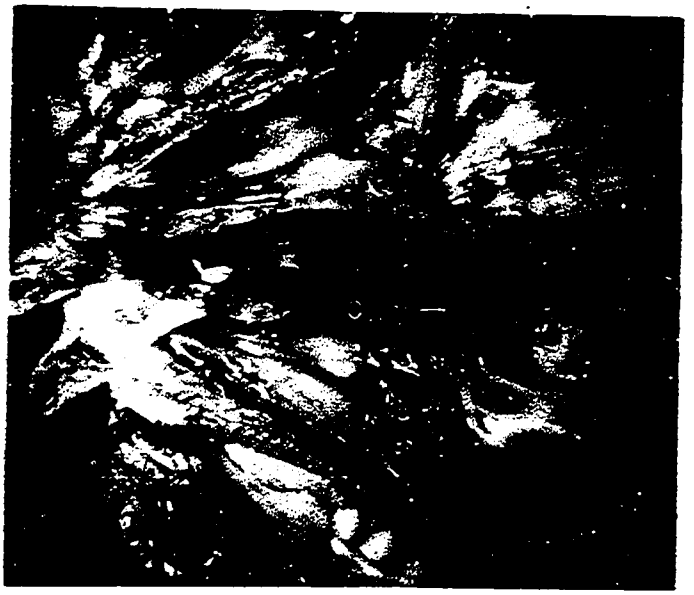


Figure 21. Mild turbinate atrophy 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate Rb-1 (rabbit origin)

Figure 22. Normal turbinates 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate D-1 (canine origin)

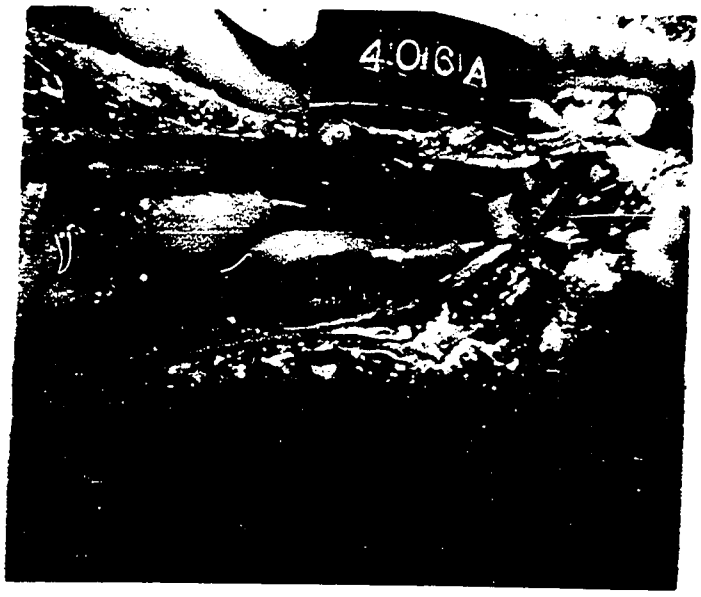

4·0161A

Figure 23. Acute pneumonia 2 weeks postinoculation in a 17-day-old pig inoculated with Bordetella bronchiseptica Isolate S-2

Figure 24. Chronic pneumonia 4 weeks postinoculation in a 31-day-old pig inoculated with Bordetella bronchiseptica Isolate S-3

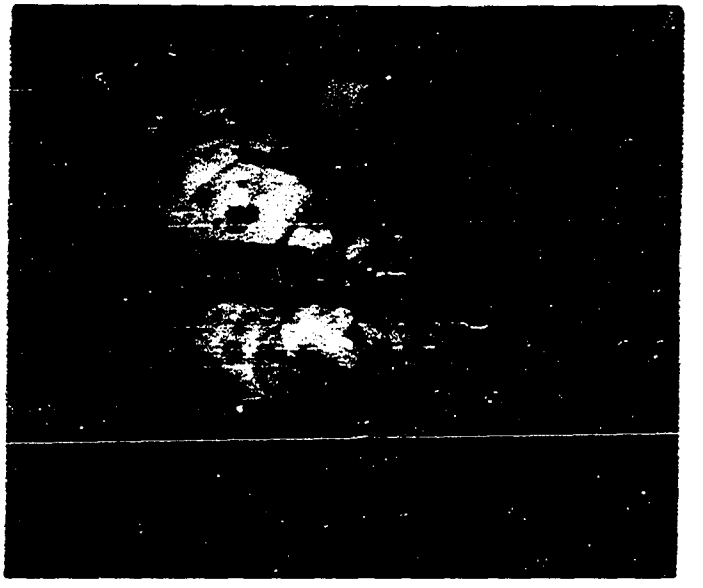


Table 9. Agglutination titers of sera collected from pigs inoculated with 8 different isolates of Bordetella bronchiseptica

Isolate	Pig No.	Plate titer	Tube titer
S-2 (swine)	1	0	0
	2	0	0
	3	0	2
S-3 (swine)	1	0	0
	2	0	0
	3	0	0
	4	0	0
S-4 (swine)	1	4	8
	2	4	8
	3	4	8
	4	0	0
S-5 (swine)	1	0	0
	2	0	0
	3	0	0
	4	0	8
D-1 (canine)	1	0	0
	2	0	0
	3	0	0
	4	0	0
Rb-1 (rabbit)	1	0	0
	2	0	2
	3	2	16
	4	0	2
Rt-1 (rat)	1	0	0
	2	0	0
	3	0	2
Ct-1 (cat)	1	0	0
	2	0	0
	3	0	0

Rb-1, Ct-1, and Rt-1) were small Gram-negative rods, sluggishly motile with peritrichous flagella and produced hemolysis on 5 percent bovine blood agar after incubation for 24 hours at 37 C. The zone of hemolysis increased in size after incubation at room temperature for an additional 24 hours. These isolates were citrate positive in 12 to 24 hours, urease positive in 2 to 12 hours and alkalinized litmus milk and dextrose in 24 to 48 hours. They did not produce indole or hydrogen sulfide. None of the isolates liquefied gelatin. They were all catalase and oxidase positive. Isolates Ct-1, Rt-1 and Rb-1 reduced nitrates to nitrites while the other 5 isolates did not.

A second group of 55 isolates were compared. Twenty-two of these isolates were from the nasal cavities of the pigs sampled in Survey I. Fifteen isolates were from the nasal cavities of pigs submitted to the Iowa Veterinary Diagnostic Laboratory for various reasons. Eight isolates were from the nasal cavities of pigs from herds with known cases of Bordetella bronchiseptica rhinitis[1]. Another group of 8 isolates were isolated between 1954 and 1956 from the nasal cavities of pigs with rhinitis. These isolates had been initially identified as Alcaligenes fecalis-like organisms[1]. One isolate was recovered from a canine lung about 1953. Another isolate was recovered from a rabbit lung about 1953.

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[1]Appreciation is expressed to Dr. W. P. Switzer who recovered these isolates.

These 55 isolates were all small Gram-negative rods. Fifty-four of the isolates were sluggishly motile. One was nonmotile. They were all citrate positive in 12 to 24 hours, urease positive in 2 to 12 hours and alkalinized litmus milk and dextrose in 24 to 48 hours. Fourteen of the 55 isolates reduced nitrates to nitrites.

All of these isolates produced hemolysis on 5 percent horse, sheep and rabbit blood after 24 hours of incubation at 37 C. Incubation for an additional 24 hours at room temperature resulted in an increase in size of the zone of hemolysis on the horse blood agar. Eight of the 55 isolates produced hemolysis on 5 percent bovine blood agar. These 8 isolates were evenly distributed among the older and more recent isolates.

DISCUSSION

Incidence Surveys

Bordetella bronchiseptica, as indicated by the data obtained from the surveys of field swine, is widely distributed among Iowa swine herds. The herd incidence of *Bordetella bronchiseptica*, as well as that of *Hemophilus spp.* and *Pasteurella multocida*, in Surveys I, II, III and IV is summarized in Table 10.

Table 10. Incidence of *Bordetella bronchiseptica*, *Pasteurella multocida* and *Hemophilus spp.* in the nasal cavities of Iowa swine

Survey	*Bordetella bronchiseptica*	*Pasteurella multocida*	*Hemophilus spp.*
Survey I (87 herds)			
No. herds positive	47	5	42
Percent herds positive	54	5	48
Survey II (87 herds)			
No. herds positive	33	8	20
Percent herds positive	38	9	23
Survey III (32 herds)			
No. herds positive	18	0	5
Percent herds positive	56	0	16
Survey IV (28 herds)			
No. herds positive	19	3	3
Percent herds positive	68	11	11

Bordetella bronchiseptica was found in 54 percent of 87 purebred swine herds sampled in Survey I. The animals sampled in this survey had been selected by their owners as being healthy, of good conformation and capable of better than average performance. Purebred pigs such as these which are sold as replacement breeding stock undoubtedly serve to disseminate Bordetella bronchiseptica among other purebred and commercial swine herds.

Bordetella bronchiseptica was found in 38 percent of 87 herds sampled in Survey II. This lower incidence as compared to Survey I may be due to the fact that the average sample size in this survey was less than 2 pigs per herd. In addition, the pigs sampled in Survey II varied from 1 to 16 weeks of age while the pigs sampled in Survey I were 8 to 10 weeks of age.

The incidence of turbinate atrophy in pigs necropsied in Survey II (26 percent of 124 pigs) was lower than that found in a similar survey conducted in the Iowa Veterinary Diagnostic Laboratory 13 years earlier. During a 6-week period Bennett (1952) found that 41.5 percent of 142 pigs submitted over 3 weeks of age had turbinate atrophy.

The high incidence of Bordetella bronchiseptica in pigs with rhinitis is demonstrated by the results of Surveys III and IV. Most of the pigs sampled in both surveys were from herds with clinical signs of rhinitis. Additional evidence associating Bordetella bronchiseptica with turbinate atrophy

can be obtained by comparing the results of Surveys II and IV. The pigs sampled in Survey II were submitted to the Iowa Veterinary Diagnostic Laboratory for many reasons while the pigs sampled in Survey IV were submitted from herds specifically suspected of having rhinitis. In Survey II, 28 percent of the herds with Bordetella bronchiseptica had pigs with turbinate atrophy and in Survey IV, 68 percent of the herds with Bordetella bronchiseptica had pigs with turbinate atrophy.

Reports from 2 other areas of the United States indicate that Bordetella bronchiseptica is frequently isolated from cases of atrophic rhinitis. In Indiana, Cross and Claflin (1962) isolated the organism from 9 of 10 pigs submitted from 10 different herds with atrophic rhinitis. A diagnostic laboratory in Georgia recently reported that the organism was frequently isolated from the nasal cavities of pigs with rhinitis (Bedell and Miller, 1964).

The presence of Pasteurella multocida in approximately 5 percent of the herds sampled is in agreement with reports by several other workers. Radtke (1938) found this organism infrequently in the nasal cavities of German swine. Shuman et al. (1953) found Pasteurella multocida in the nasal cavities of only 6 of 55 pigs in a herd with rhinitis. Heddleston et al. (1954) isolated the organism from the nasal cavities of 8 percent of the pigs with rhinitis and 4.3 percent of the normal pigs in 2 herds with atrophic rhinitis. Olander (1961) isolated Pasteurella-like organisms from the

nasal cavities of 5 of 69 slaughter-weight pigs in California.

These findings are not in agreement with reports by Canadian workers that Pasteurella multocida was frequently isolated from the porcine nasal cavity. MaKay (cited by Schofield and Jones, 1950) found Pasteurella multocida in the nasal cavities of 65 of 75 pigs with atrophic rhinitis. Jones (1952) observed that the organism was rarely absent from the early lesions of atrophic rhinitis and Gwatkin et al. (1953) reported that in 186 bacteriologic examinations this organism was present in the nasal cavities of 38 percent of the pigs with turbinate atrophy and 16 percent of the pigs with normal turbinates.

The results of studies on Canadian swine and limited observations by the author indicate that a high percentage of the pigs in some herds have nasal infection with Pasteurella multocida. Such pigs have well defined clinical signs of rhinitis and may remain infected for extended periods of time. Strains of Pasteurella multocida involved in such cases appear to have an unusual affinity for the nasal cavity. Possibly most strains of the organism have a lower affinity for the nasal cavity and are recovered from that area because of continual reinfection from pneumonic lungs.

Pneumonia was observed in 43 percent of the pigs in **Survey II.** Culture of the pneumonic lungs of 35 of these pigs revealed that 10 had Pasteurella multocida and 2 had Bordetella bronchiseptica. Culture of the nasal cavities of

the same pigs revealed that 10 had Bordetella bronchiseptica and 5 had Pasteurella multocida. These findings indicate that Bordetella bronchiseptica is more common in the swine nasal cavity and Pasteurella multocida is more common in the pneumonic swine lung. This is in agreement with L'Ecuyer et al. (1961) who found Pasteurella multocida and Bordetella bronchiseptica in 40 percent and 1 percent respectively of 86 pneumonic swine lungs. Similar findings on the incidence of these 2 organisms in pneumonic swine lungs were reported by Spray (1922), Thorp and Tanner (1940), and Morcos et al. (1947).

Investigations in this laboratory indicate that approximately 50 percent of Iowa swine have pneumonia when slaughtered. The high incidence of Pasteurella multocida in pneumonic swine lungs and the high incidence of pneumonia in swine indicate that this organism is extremely common on a herd basis in Iowa swine.

The finding in Survey I that Hemophilus spp. were present in 48 percent of the herds is in reasonable agreement with Radtke (1938) who found that 60 to 70 percent of German swine harbored the organism in their nasal cavities. Braend and Flatla (1954) found these organisms in the nasal cavities of many Norwegian swine. Olander (1961) isolated Hemophilus spp. from the nasal cavities of only 6 of 69 market-age swine in California.

Hemophilus spp. were isolated from 23 percent, 16 percent

and 11 percent of the herds in Surveys II, III and IV respectively. The lower incidence could be a reflection of variability in age of pigs sampled and small sample size in comparison to Survey I.

Transmission Trials

The clinical appearance of sneezing correlated to a high degree with the presence of catarrhal exudate on the nasal mucosa of experimentally infected pigs. In general, sneezing and catarrhal exudate were observed from the fifth day to the second or third week postinoculation. Sneezing was rarely observed after 3 weeks postinoculation in any of the trials and the surface of the nasal mucosa appeared to have a normal coating of secretions in most pigs necropsied after this period. However, culture of these secretions routinely revealed abundant growth of Bordetella bronchiseptica until after the twelfth week postinoculation.

In some cases of natural Bordetella bronchiseptica rhinitis, sneezing and catarrhal rhinitis were observed in pigs up to 6 or 8 weeks of age. Possibly these pigs were infected at a later age and were in the acute stages of infection at the time of necropsy. This is quite conceivable since weaning and pooling of larger groups of pigs commonly occurs at this age in field swine. In herds with only a few carrier sows, many pigs might remain uninfected until they came into contact with other infected pigs at weaning time.

Observations made in this laboratory indicate that this frequently occurs.

A higher incidence of sneezing was observed in pigs inoculated at 4 weeks of age in Trial I than in pigs inoculated at 3 days of age in Trials II and III. These results and similar observations made in other trials in this laboratory indicate that the sensory apparatus of the nasal mucosa of the 4-week-old pig may be better developed than that of the 3-day-old pig. Additional support for this concept is gained from observations made by the author that sneezing associated with Bordetella bronchiseptica, Pasteurella multocida or Hemophilus spp. infection in 1- to 3-week-old field pigs was relatively mild as compared with that seen in older pigs from which these organisms were isolated. The increased incidence of sneezing in weanling pigs affected with atrophic rhinitis is generally attributed to increased severity of the lesions. However, it is possible that increased sensitivity to irritation also contributes to this increase in sneezing.

In Trial I significant turbinate atrophy was not detected until 3 weeks postinoculation. In Trial II moderate turbinate atrophy was observed in all 3 pigs necropsied at 2 weeks postinoculation. Similar lesions were observed in 3 pigs that died about 2 weeks postinoculation in Trial III. In addition, in Trial II the lesions became progressively more severe from the second week to the fifth week postinoculation when 7 of 10 pigs necropsied had severe turbinate atrophy. On the other

hand, in Trial I the older pigs appeared to resist the effects of the infection since neither the incidence nor the severity of the lesions increased from the third week to the eighth week postinoculation. These findings are consistent with the concept that older swine are less susceptible to atrophic rhinitis than younger swine (Jones, 1947).

Turbinate atrophy observed in pigs experimentally infected with Bordetella bronchiseptica was as severe as that observed in the majority of field pigs with atrophic rhinitis. In many cases of atrophic rhinitis in field pigs, Bordetella bronchiseptica was isolated in abundant growth with virtually no other bacteria detected on modified MacConkey's agar or 5 percent bovine blood agar. In all respects, the field disease closely simulated the experimental disease.

The results of Trial III indicate that many isolates of Bordetella bronchiseptica are pathogenic for young pigs. The ability of isolates of the organism from a rabbit, a cat and a rat to cause rhinitis and pneumonia in pigs clearly shows that these animals may serve to infect swine herds with the organism. Rats, cats and dogs are frequently in close habitation with swine in confinement. Reports by several workers have implicated rats, cats, dogs and rabbits as carriers of atrophic rhinitis (Thunberg and Carlstrom, 1946; Jones, 1947; Schofield, 1955; Gwatkin and Dzenis, 1955; Andrews et al., 1957). Pasteurella multocida was present in the material transmitted from the various species to swine in some cases.

However, it is quite possible that Bordetella bronchiseptica was also present.

Culture of the nasal exudate of pigs necropsied at various intervals up to 12 weeks postinoculation revealed large numbers of Bordetella bronchiseptica. From the twelfth to the sixteenth week postinoculation, the number of organisms recovered from each of 11 pigs in Trial II decreased. By the twentieth week postinoculation, only 2 of the 11 pigs harbored the organism in their nasal cavities. Switzer (1963) made similar observations on the elimination of the organism from the nasal cavities of swine as they matured.

Samples of nasal exudate collected from live pigs at various intervals were usually positive for the organism. However, all samples collected from 15 pigs at 7 days postinoculation were negative in Trial I. Samples collected from 3 penmates at necropsy were positive. It is suspected that the organism was not disseminated throughout the nasal cavity and, as a result, the sampling technique was inadequate.

Intranasal inoculation of 3 pregnant sows in Trial II did not result in infection. These sows were constantly exposed to infected pigs, but remained negative when samples of nasal exudate were cultured. These animals were not necropsied, so it is possible that more exhaustive sampling of the nasal secretions might have detected the organism. It has been found that some naturally infected sows harbor the organism in only 1 nasal passage (Switzer, 1963).

The elimination of the organism from the nasal cavity and the apparent regeneration which occurred in Trial II indicate that some change occurred in the host or the organism. This subsequently resulted in the elimination of the organism from a high percentage of animals which were previously quite heavily infected.

The factor responsible for elimination of Bordetella bronchiseptica from the nasal cavity could be specific antibody, a nonspecific resistance factor such as an increase in lysozyme or some other inhibitory factor liberated in the nasal secretions, or it could be a mutation or phase change in the organism. It seems doubtful that circulating antibody would have any appreciable effect on nasal infection in swine with Bordetella bronchiseptica since high titers of circulating antibody in rabbits have been shown to be without effect on nasal infection with the organism (Bailey, 1927). In addition, the circulating antibody titers in infected pigs were quite low. It is more conceivable that a local antibody or resistance factor is produced by the mucosal cells of the nasal cavity. The possibility of a phase change in the organism should be considered since Lacey (1953) has shown that changes in growth temperature and growth medium composition can induce such changes in this organism. Organisms altered in their growth characteristics in this manner might find the environment in the maturing swine nasal cavity less desirable. In some instances, rough colony forms were

observed in primary cultures from infected pigs. The significance of these is not known.

Bordetella bronchiseptica was isolated in heavy growth from the tracheal secretions of all pigs necropsied in Trial II from 1 through 5 weeks postinoculation. In Trial I the pigs began eliminating the organism from the trachea about 4 weeks postinoculation. Culture of tracheal exudate from 3 pigs necropsied 8 weeks postinoculation in this trial revealed no Bordetella bronchiseptica.

In all 3 trials coughing was observed in the inoculated pigs. Lesions of pneumonia were observed in some of these pigs; however, necropsy of the pigs in Trial I as well as many pigs in Trials II and III which had been observed to cough revealed no pneumonia. It was observed that pigs with pneumonia had coughed more frequently and for a longer duration. Catarrhal exudate was observed in the tracheas of most pigs and the organism was consistently recovered from this exudate up to 4 to 5 weeks postinoculation. The presence of this organism in the tracheas and bronchi of infected pigs apparently caused irritation with resultant coughing. Since very little evidence of inflammation was observed in the tracheas of these pigs, it appears that the organism was liberating a toxic substance which then caused the coughing. It seems reasonable that the mechanism may be similar to that seen in whooping cough in man.

Bordetella bronchiseptica can survive in apparently

normal swine lungs for a considerable time. Four pigs necropsied at 4 different intervals (1 week, 2 weeks, 3 weeks, and 6 weeks postinoculation) in Trial I and 3 pigs necropsied at 3 different intervals (1 week, 2 weeks, and 5 weeks post-inoculation) in Trial II had the organism in apparently normal lung tissue. In Trial III, 2 pigs inoculated with Isolate S-4, 1 pig inoculated with Isolate Ct-1, and 1 pig inoculated with Isolate Rt-1 had the organism in normal lung tissue at 4 weeks postinoculation.

The presence of this organism in these normal lungs is undoubtedly associated with heavy growth of the organism in the trachea and bronchi. Stress or exposure could probably precipitate a severe pneumonia in such animals. The work of Phillips (1943), Ray (1950, 1959), L'Ecuyer (1961), Dunne et al. (1961), and Goodwin and Whittlestone (1964) indicate that this frequently occurs. These reports also indicate that Bordetella bronchiseptica has been widely distributed among swine for many years. The organism was isolated from the nasal cavities of pigs with a rhinitis in Illinois almost 25 years ago (Daugherty, 1941).

The apparent failure of many investigators to associate this organism with atrophic rhinitis may be due to several factors. The organism is slow to develop on culture media and is frequently overgrown by other organisms that are common in the porcine nasal cavity. The organism is frequently not detected unless cultures are incubated at least 48 hours.

The organism was confused with Alcaligenes fecalis in earlier work (Switzer, 1956; Claflin, 1958). A third possibility is that culture of nasal exudate was not made from pigs during the early stages of infection. In advanced cases of turbinate atrophy this organism is frequently crowded out by other bacteria.

The presence of agglutinating antibodies in preinoculation serum samples from the 3 sows used in Trial II raised doubt about the specificity of the agglutination test employed. The herd from which these sows were obtained has been repeatedly negative for Bordetella bronchiseptica on culture of nasal secretion and the sows themselves were negative for the organism in all preinoculation and postinoculation nasal exudate cultures. The prevalence of low levels of agglutinating antibodies in sera collected from field swine of various ages indicated that these antibodies are quite common in adult swine.

Possibly the antibody detected in the sera from these sows was present due to antigenic stimulation by some organism with antigens common to Bordetella bronchiseptica. Antibody titers of 1:64 that developed by the eighth week postinoculation in Trial I probably were specific since uninoculated controls did not develop these titers. Although further elucidation of the value of the tests employed in this work is needed, it appears that titers of 1:32 or higher may be significant while those below should be disregarded in view of

the possibility of nonspecificity.

The delayed development of these antibodies is similar to the appearance of antibodies in pertussis in humans. Lautrop and Lacey (1960) state that in Bordetella pertussis infections in man, specific antibodies appear in the blood between the third and fourth week and reach a maximum about the seventh to eighth week of the disease.

SUMMARY

The incidence of Bordetella bronchiseptica in Iowa swine was studied in 4 different surveys. The organism was isolated from the nasal cavities of pigs in 54 percent of 87 purebred swine herds in Survey I. The herd incidence of the organism was 38 percent in a second survey of 87 commercial and purebred herds.

Bordetella bronchiseptica was found in a higher percentage of herds with clinical signs of rhinitis in Surveys III and IV. The organism was isolated from 56 percent (18/32) and 68 percent (19/28) of the herds in Surveys III and IV respectively.

Hemophilus spp. and Pasteurella multocida were found in 48 percent and 5 percent of 87 purebred herds respectively. Comparable findings on the incidence of these organisms were made in the other 3 surveys.

Intranasal inoculation of Bordetella bronchiseptica in 4-week-old pigs resulted in turbinate atrophy in 10 of 15 pigs necropsied from 2 to 8 weeks postinoculation. Intranasal inoculation of the organism in 3-day-old suckling pigs resulted in turbinate atrophy in 15 of 16 necropsied from 2 to 5 weeks postinoculation. Randomly selected littermates necropsied at 20 weeks postinoculation had regenerated turbinates that occupied the normal amount of space in the nasal cavity. The organism was recovered in abundant growth from

the nasal exudate of all experimentally infected pigs up to 12 weeks postinoculation. It gradually disappeared from the nasal cavities of the older pigs so that only 2 of 11 pigs had the organism in their nasal cavities at 20 weeks of age.

Four different isolates of Bordetella bronchiseptica recovered from nasal exudate of pigs from herds with atrophic rhinitis produced moderate turbinate atrophy in box-reared pigs inoculated at 3 days of age. Similar pigs inoculated with isolates of the organism recovered from tracheal exudate of a cat, tracheal exudate of a rat and nasal exudate of a rabbit also developed turbinate atrophy. One isolate of Bordetella bronchiseptica recovered from nasal exudate of a puppy with canine distemper produced no evidence of respiratory disease in these box-reared pigs.

Bordetella bronchiseptica was isolated in heavy growth from the tracheal exudate of many of the experimentally infected pigs. Its presence in the trachea and bronchi frequently elicited coughing during the early stages of infection. Many of the box-reared pigs also developed pneumonia.

The organism was isolated from the normal lung tissue in about 15 percent of the inoculated pigs. Survival in this tissue for extended periods of time is probably associated with growth of the organism on the tracheal and bronchial mucosa. There is undoubtedly a very close relationship between the presence of this organism in the trachea and bronchi and the severe pneumonia caused by the organism in experimentally and

naturally infected pigs.

Sixty-three isolates of Bordetella bronchiseptica were found to be identical in their ability to produce urease, alkalinize litmus milk and dextrose and utilize citrate. Seventeen of the 63 isolates reduced nitrates to nitrites.

The agglutinating antibody response of pigs infected with Bordetella bronchiseptica is relatively protracted and weak. Nonspecific agglutinating antibodies in low titer appear to be quite common in adult swine.

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